Award Number: W81XWH-12-1-0243

TITLE: Temporal Progression of Visual Injury from Blast Exposure

PRINCIPAL INVESTIGATOR: Brittany Coats, PhD

CONTRACTING ORGANIZATION: University of Utah
Salt Lake City, UT 84112

REPORT DATE: September 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2016	Annual	1 Sep 2015 – 31 Aug 2016

4. TITLE AND SUBTITLE

Temporal Progression of Visual Injury from Blast Exposure

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0243

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Brittany Coats, Daniel Shedd

E-Mail: bcoats@eng.utah.edu; d.shedd@utah.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

UNIVERSITY OF UTAH, THE
201 S PRESIDENT CIRCLE RM 408
SALT LAKE CITY UT 84112-9023

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this grant is to investigate the temporal progression of eye injury from blast exposure and identify early predictors of visual dysfunction. The studies performed in the previous year have shown that blast exposure (approx. 225 kPa magnitude) in a rat model leads to time-dependent ocular pathology changes over the course of eight weeks. Specifically, we have found that the behaviorally assessed visual acuity of blast exposed animals is significantly degraded following blast exposure. The decrease in visual ability is statistically significant when comparing blast-exposed animals to their baseline, pre-blast visual ability results. The decrease is also significant when comparing control and blast exposed animals at each time point after exposure. These deficits first become significant at two weeks after blast, and do not resolve by the end of the study. The visual acuity findings appear to be initially attributed to immediate retinal damage following blast exposure, but corneal injury also contributes to vision degradation several weeks after the initial blast exposure. We also found early biomarkers of corneal damage that could lead to treatment opportunities for corneal scarring.

15. SUBJECT TERMS
Biomechanics, ocular trauma, blast, rats, visual acuity

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	44	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

INTRODUCTION

Ocular trauma during military conflicts has steadily increased from 0.5% in the civil war to 13% in present day. This increase is likely associated with the advancement of weaponry and the increased use of explosive devices. The majority of eye injuries from an explosion can be classified as either open globe or closed globe. Open globe injury is often readily identifiable and typically undergoes urgent surgical repair. However, closed globe injury may not be detected immediately and can result in a series of sequelae that lead to visual dysfunction months after the blast. The progression of closed globe eye injury and visual degradation following blast exposure has not been well characterized. Furthermore, it is unknown if there are early indicators that denote an increased risk for developing visual dysfunction following blast exposure. Therefore, the objectives of this proposal are to investigate the temporal progression of eye injury from blast exposure and identify early predictors of visual dysfunction. We propose to accomplish these objectives by first identifying the probability of military personnel developing visual system injury after blast exposure, and determining the time point after blast exposure that visual system injury becomes identifiable. Next, we propose to systematically evaluate the time course of visual system injury from blast exposure using our existing rat model for blast traumatic brain injury. From these experimental studies we can identify early predictors of visual dysfunction. Finally, we will evaluate these early predictors in a clinic setting to verify their usefulness in real-world scenarios. By understanding the temporal and chemical progression of eye injury from blast exposure, we can establish early identifiers of visual system injury. This will enhance our diagnostic capabilities and lead to the development of time-dependent treatment strategies to mitigate the loss of vision in military personnel.

KEYWORDS: blast, vision loss, biomarkers, pressure, ocular trauma, animal model, clinical study

OVERALL PROJECT SUMMARY

Aim 1: Investigate the progression of visual system injury in service members exposed to a blast.

Current Objectives

- Statistically determine the time after the blast exposure that visual dysfunction is identifiable. (SOW 3)
- Identify local cases of military personnel with exposure to blast injury and no identifiable signs or symptoms of visual injury. (SOW 4)
- Perform a prospective study on blast exposed service members. (SOW 4)

Key Methodology

University of Utah health records were searched for the following ICD9 codes: E993, E921, E923, E803, E837, E993.4, E890.0, E923.9. These ICD9 codes involve injuries from multiple types of explosions. The target date range was from 2005 to present. Inclusion criteria for this study are (1) No obvious sign of open globe trauma (facial burns, shrapnel to the eye, etc.) (2) Eye examination following blast exposure. Our control group consists of people involved in other traumatic injuries that would not affect the visual system (e.g., accidental or inflicted trauma to the extremities or torso without an associated head impact).

Medical records will be evaluated for information that may provide insight into the severity of the blast. Any history associated with the blast exposure will be investigated for signs of stand-off distance, height of the explosive, and the type of the explosive. In addition, injuries related to the initial blast exposure will be identified and given an assessment score based on the Abbreviated Injury Scale (AIS) which is an anatomical scoring system for classifying the severity of the injury. An increased injury severity score will be assumed to indicate an increased severity of blast exposure. We found limited ophthalmology medical data available for many of the veterans in our database. Therefore, we have partnered with the Vision Center of Excellence to obtain data from the Departments of Defense and Veterans Affairs ocular care clinical data repository DVIEVR. Our request has been approved and we are waiting for the data. We have also gotten IRB approval to advertise for our prospective study in the Student Veterans Support Center at the University of Utah. The flyer used for recruitment is attached as **Appendix A**.

All statistical analyses will be performed using SAS statistical software (JMP 10.0, Cary,NC). Descriptive and univariate analyses will first be performed to identify the occurrence of delayed visual system injury after blast exposure. Of the cases with delayed visual system injury, the time between the blast exposure and diagnosis will be collected. Significant differences with age, gender, the presence of absence of traumatic brain injury, and blast severity will be evaluated. Statistical significance will be set

at a p-value of < 0.5. Logistic regression will also be used to determine the probability for developing visual system injury following blast exposure given age, gender, blast severity, and the presence/absence of traumatic brain injury. In addition, a survival analysis will be performed using Cox's proportional hazards regression model to determine the time post blast exposure that visual system injury is most likely to be identified. Multiple regression analysis will be used to determine the effect of participant age, gender and blast severity on the survival analysis.

Results

Two Health Data Analysts replaced medical students for data capture. One of the employees already had VA clearance, the other did not. After receiving approvals from IRB, training, and getting access to the appropriate databases (all achieved after mid-November 2015), they began extracting data from the VA medical record system.

The VA retrospective data extraction has been completed. **Table 1** provides a brief summary of the population characteristics and findings from the data. From this table, it is interesting to note that the majority of VA patients (53.1%) evaluated had multiple blast exposures. Photophobia was a common complaint among the population (79.8% of blast exposed veterans).

Forty veterans from our retrospective study have been selected for invitation to our prospective study. These veterans have had a blast exposure without diagnosis of a TBI. We are interested in detecting subtle changes in the eye without complications of brain injury. However, even without a diagnosis, the presence of mild TBI is likely. Therefore, in our next round of recruitment, we will target those with a mild TBI diagnosis. We have also gotten approval to recruit through the University of Utah Student Veteran's Support Center. This population of students will capture veterans without TBI or polytrauma that may complicate interpretation of the results. The recruitment flyer for the student center is attached to this report. A no cost extension for one year was requested to complete the clinical studies. This extension was approved. To date, 10 veterans have expressed interest in participation of our study.

Progress and Accomplishments

All IRB and HRPO approvals are obtained. Data extraction from the SLC VA was completed. We have selected and contacted forty veterans for initial inclusion into the prospective study, and began recruitment at the University of Utah Student Veterans Support Center. We are collaborating with the Vision Center for Excellence, and applied for additional retrospective data from the DVIEVR database. We are currently training staff to perform the specific visual examinations, and plan to start prospective studies in October 2016.

Table 1. Summary of retrospective VA population and blast exposure characteristics.

Gender		
	Male	98.0% (287)
	Female	1.7% (5)
Age		
	Mean	30.96±6.73
	Median	29
	Min	21
	Max	58
Race		
	American Indian/Alaska Native	0.7% (2)
	Asian	0.7% (2)
	African American	1.7% (5)
	Hispanic or Latino	7.3% (21)
	Native Hawaiian/Pacific Islander	1.4% (4)
	Caucasian	86.5%(249)
	Unknown	1.7% (5)
Blast Mechanism		
	Improvised Explosive Device	68.9%(202)
	Rocket Propelled Grenade	19.5% (57)
	Mortar	21.5% (63)
	Other	4.4% (13)
	Unknown	6.5% (19)
No. of Blast Exposures		
	1	46.9%(134)
	2-5	33.9% (97)
	>5	10.1% (29)
	Unknown, but >1	9.1% (26)
TBI Diagnosis		
	No	16.4% (48)
	Yes w/out Behavioral Health Issues	26.4% (77)
	Yes w/ Behavioral Health Issues	56.5%(165)
	No Diagnosis Recorded	0.7% (2)
Vision Complaint Severity (blurring, troubles seeing, etc)	None	23.3% (66)
	Mild	33.2% (94)
	Moderate	29.0% (82)
	Severe	10.6% (30)
	Very Severe	3.9% (11)
Photophobia	None	20.1% (57)
	Mild	24.0% (68)
	Moderate	26.5% (75)
	Severe	21.2% (60)
	Very Severe	8.1% (23)

Aim 2: Investigate the progression of visual system injury following blast exposure in an animal model and identify early indicators of visual dysfunction.

Current Objectives

- Complete histology for ocular injury from blast exposure. (SOW 3)
- Disseminate research findings to the public and research communities.

Key Methodology

Briefly, adult Long Evans rats (n=64) were administered carprofen one day before the blast for pain management. A baseline of vision functionality was established before the blast using the custom optokinetic tracking device we developed in Year 1 (**Figure 1A**). For increased accuracy, each animal was tested three times on each testing day and an average acuity was used for the final measurement. In Year 4, we updated our behavior code to separately assess visual ability in each eye.

On the day of the blast, the experimental (n=64) and control (n=38) animals were anesthetized using inhaled isoflurane followed by an injection of ketamine and dexmetomedine administered IP. The animals were then placed in a custom rat holder designed in to provide a side-on blast exposure while preventing injury to the animal torso (**Figure 1B**). Only experimental animals were exposed to a blast. Control animals were removed from the device after 30 seconds. After removal from the blast device, all animals were allowed to recover from the anesthesia and returned to the animal facility.

Animals did not show signs of pain following the blast exposure, but carprofen was administered the next day as a precaution. Vision metrics (vision behavior, OCT) were repeated the day after the blast and every subsequent week following the blast until sacrifice. At sacrifice, the eyes and brain were harvested for histological analysis. Because the temporal changes of injury were ongoing at 4 weeks, the length of the survival period was increased to 8 weeks and only a single blast level evaluated.

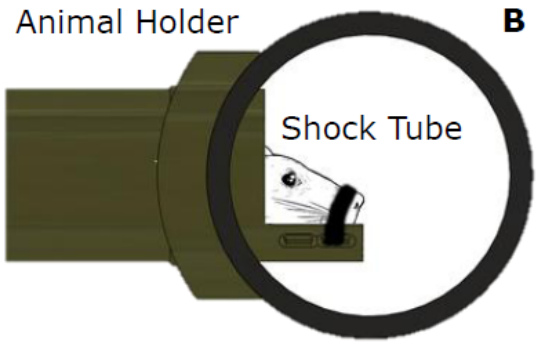


Figure 1. (A) Custom optokinetic device designed to test visual acuity in rats. (B) Rat mounted in the shock tube for side-on blast exposure.

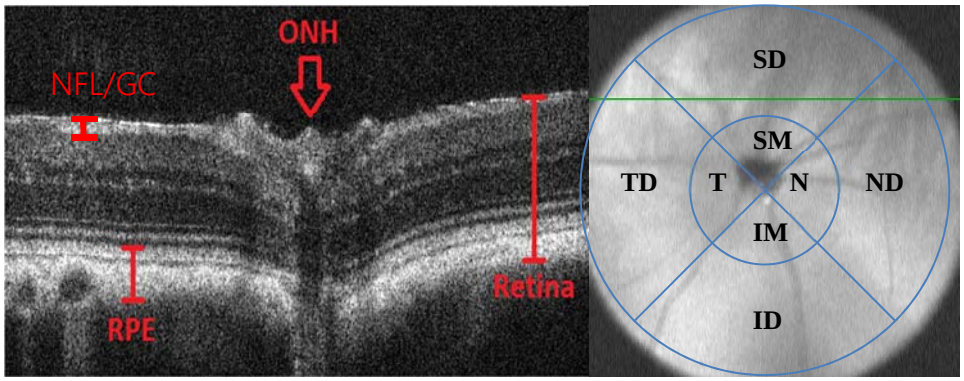


Figure 2. (A) Optical coherence tomography was used to monitor changes in the retinal thickness. (B) The thickness of the RPE and retina were averaged across regions defined by a radial pattern around the optic nerve of the eye. SM/SD (superior medial/distal); IM/ID (inferior medial/distal); NM/ND (nasal medial/distal); TM/TD (temporal medial/distal)

Retinal and corneal thickness were measured over time using optical coherence tomography and two custom MATLAB image processing programs. The retina image processing program measured the thickness of the RPE and NFL/GC retinal layers, as well as the overall retinal thickness (**Figure 2A)**. Thickness was determined at forty locations across the 2D image and averaged. The average for each image was then averaged with other images in the same retina region. The regions were defined in a radial pattern relative to the optic nerve as illustrated in **Figure 2B**: superior medial/distal (SM/SD), inferior medial/distal (IM/ID), nasal medial/distal (NM/ND), and temporal medial/distal (TM/TD). Corneal thickness measurements were initially automated, but we switched to manual measurements of the stromal, epithelial, and overall corneal thicknesses to better identify and capture injured and scarred regions of the cornea.

In a small subset of the blast rats (n=10), intraocular pressure (IOP) was directly measured during the blast exposure. To achieve this, micro pressure transducers were pre-threaded through surgical tubing and an 18G hypodermic needle post. The needle post and pressure transducer was inserted through the posterior sclera and positioned in the central vitreous chamber. Skin sutures and cyanoacrylate adhesive were used to secure the surgical tubing to the rats head, and prevent motion relative to the eye during blast wave impact. Placement of the sensors in the eye, and an example intraocular pressure trace are provided in **Figure 3**.

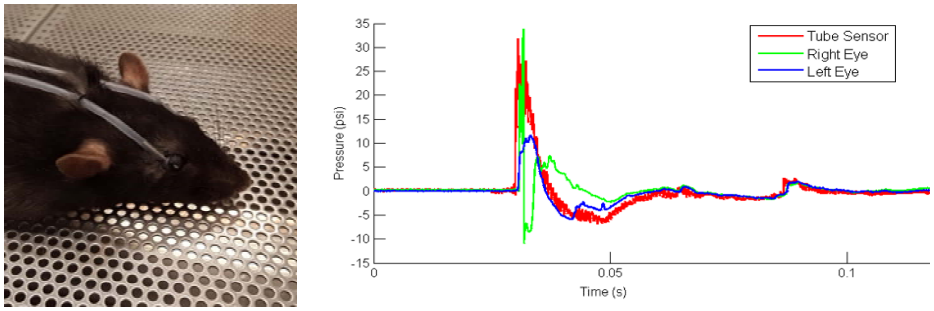


Figure 3. (Left) IOP sensors pass through needles into the vitreous space. (Right) Pressure traces of blast pressure in the shock tube and inside each eye.

Results

The mortality rate of all animals exposed to a blast was 6.3% (4/64). One or two of these deaths may have been related to anesthesia complications, but no deaths occurred in the control groups. There was a significant initial drop in visual acuity in rats following blast exposure compared to. Over a period of 8 weeks, visual acuity decreased and never recovered to baseline levels (**Figure 4**). In fact, the visual acuity from control animals improved slightly, likely due to increased familiarity with the optokinetic device and less distraction by surroundings during testing.

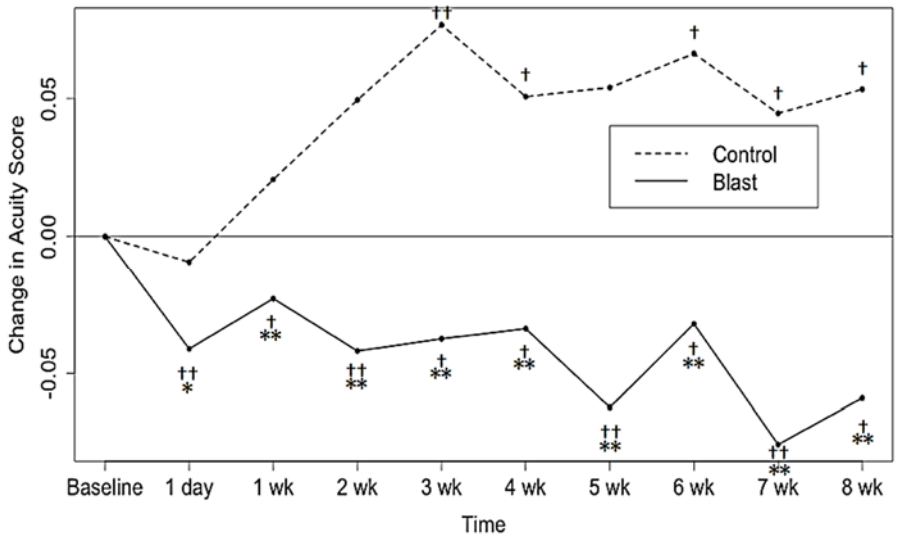


Figure 4. Visual changes after blast exposure in experimental and control animals. */**Significant differences between control and blast exposed animals at each time point. †Significant changes relative to baseline visual ability.

Two of the 10 animals used to measure IOP were excluded from analysis due to damage to the sensors. As the blast wave traveled initially through the head, IOP closely tracked the external pressure. However, after initial impact IOP readings became erratic, likely from sensor movement within the eye or impact of the lens onto the sensor tip. Based on initial IOP curve characteristics it was observed that peak pressure in the directly exposed eye matched the external pressure (within 0.1% variation), while the indirectly exposed eye experienced a 30% reduction in peak pressure ($p=0.07$) compared to the external pressure. The rate of pressurization decreased significantly as the blast wave traveled from the tube into the right eye and continued to decrease as the wave traveled through the body into the left eye ($p<0.0001$). No significant difference was observed in positive, negative, or net impulse delivered to either eye. This data suggests that peak pressure measured externally to an animal (but nearby) is likely adequate for estimating peak IOP in an eye directly exposed to the blast. However, it likely overestimates the pressure in an indirectly exposed eye, and overestimates the load rate of both directly and indirectly exposed eyes.

The corneal OCT analysis resulted in significant, time-dependent changes in overall corneal layer thickness (**Figure 5**). Stromal thickness significantly increased at one week post-injury in the eye contralateral to the blast. This thickness change resolved by week 2. In the eye ipsilateral to the blast, the stromal thickness significantly increased at week 2 and stayed increased until week 5. The epithelium significantly increased at week 5 and remained increased until week 6. This increase only occurred in the eye ipsilateral to the blast. A small subset of eyes were examined immediately post-blast using fluorescein staining to ensure that corneal injuries were not induced by sporadic particles caused by membrane rupture. We are confident that these injuries are truly caused by the blast pressure wave.

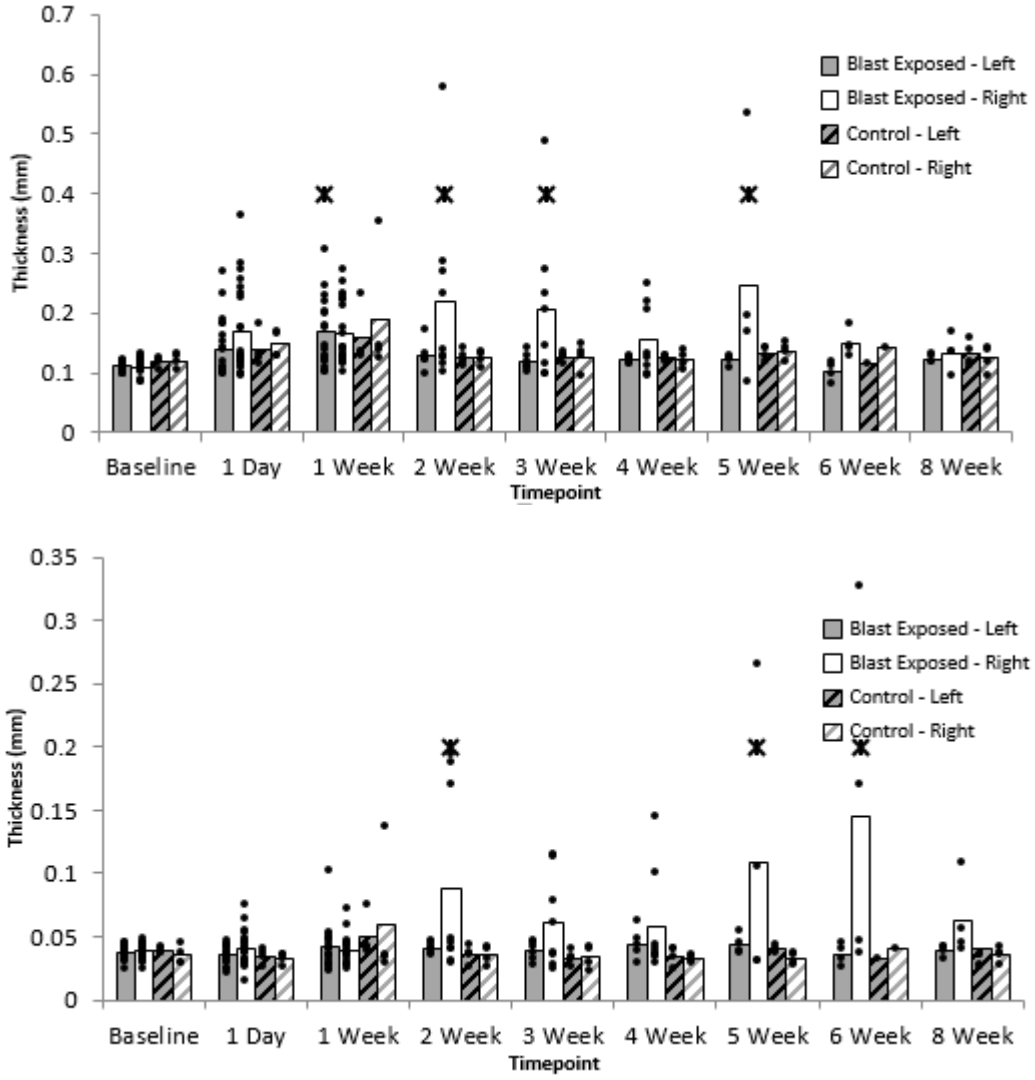


Figure 5. Thickening of the corneal stroma **(top)** and epithelium **(bottom)** as a function of time. Significance stars indicate significant changes at the .05 confidence level using Dunnett's test. Columns represent average thickness with individual thickness measurements overlaid. Stroma significantly thickened from baseline at weeks 1-3 and 5, while the epilthelium thickened at weeks 2, 5, and 6. The majority of the changes were seen in the blast exposed (ipsilateral) eye.

The retinal OCT results were not as conclusive as the corneal findings. Statistical analysis found a significant thickening of the medial retinal thickness at seven weeks in the ispsilateral eye of blast-exposed animals. There were no significant findings in other regions or time points. There were also no significant findings in any group when examining overall thickness by quadrant (temporal, inferior, nasal, superior). There were no significant thickness changes from baseline in the RPE layer in any group or time point. Finally, the analysis of the combined NFL/GC layer likewise found no significant thickness changes from baseline. Additional evaluation was performed to confirm our findings manually (**Figure 6**).

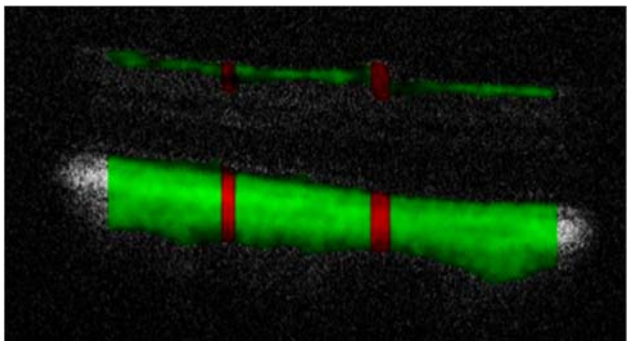


Figure 6. Output video of NFL/GC analysis program verifying automated measurements. Red areas were detected but automatically omitted from thickness measurements due to the presence of blood vessels or excessive noise.

Progress and Accomplishments

All animal testing is complete. We increased some sample sizes to increase overlap between the subsets of animals with corneal injury and those with histology. These studies are completed, and histological evaluation using H&E, TUNEL, and Wheat Germ (**Figure 7**) is in progress. We also completed the intraocular pressure studies. These studies included purchasing additional pressure transducers and continual calibration to ensure accuracy, and designing an attachment system to the eye to minimize relative motion between the sensor and the eye during blast exposure. We are currently evaluating the data and preparing a manuscript for dissemination

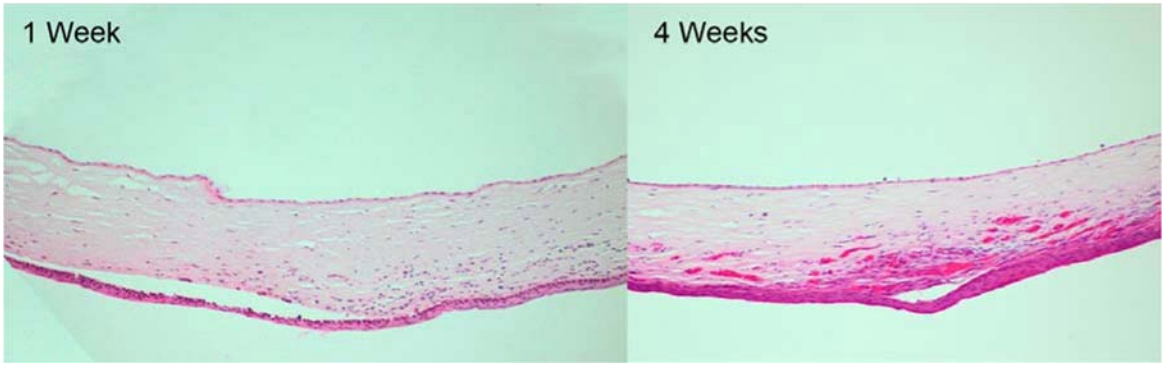


Figure 7. Increased cellular activity at 1 week (purple dots) are indicative of eventual neovascularization (bright pink regions) found at 4 weeks. Damage to Bowman's layer (separation between stroma and epithelium) is also evident.

Data extraction from the retinal and corneal images was completed, with regional and layer thicknesses both recorded. Statistical analyses on the final set of animal studies have been completed for the visual behavior studies, and cornea/retinal thickness evaluations. We have also begun work to adapt our procedures for future mouse studies. The mouse experiments will allow us to use genetic knockouts to investigate injury pathways. We developed a modified mouse holder that adapts the rat holder apparatus for the smaller body of the mouse (**Figure 8**). Several blasts have been performed on post-mortem rats to develop a technique for identifying damaged collagen in the cornea following blast exposure.

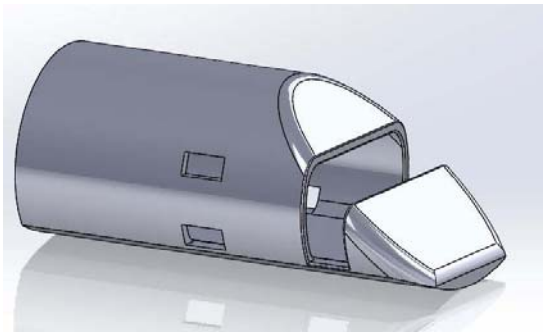


Figure 8. Mouse holder. Slot allows eye to be exposed to blast while preventing excess head motion or torso injury. Holder fits inside existing rat holder.

We published a book chapter titled "Biomechanics of Ocular Trauma in the Military." We have also drafted two journal manuscripts for upcoming submission, with the first containing development of the shock tube, IOP testing, visual acuity, and OCT results. A second publication is also being drafted regarding the histology findings and vitreous proteomic changes and following blast exposure.

Our future work will involve the development of a computational model to investigate the effect of varied eye geometry (between human and rat eyes) as well as blast parameters on the stresses and strains that develop in the eye during blast. We are particularly interested to see if high strain (or another injury metric) develops in the cornea, which could link to the findings of our animal studies.

Aim 3: Identify changes in vitreous protein expression that correlate with visual system injury

Current Objectives

- Determine significantly different levels of protein among the experimental groups and between the eye ipsilateral and contralateral to the injury in each animal. (SOW 3)
- Compare levels of protein expression to visual acuity, retinal thickness changes, and positive histopathology to identify potential biomarkers for diagnosis of eye injury. (SOW 3)
- Disseminate results to the public and research communities.

Key Methodology

The vitreous from half of the animals in each group at 1 day and 1, 4 and 8 weeks post-blast were evaluated for biomarkers of ocular trauma. VEGF and other cytokines were measured using a commercially available antibody array (RayBio Rat Cytokine Antibody Array G, RayBiotech, Norcross, GA). Signal intensities were evaluated using an ELISA plate reader at an excitation frequency of 532 nm. Positive and negative controls in the array allowed comparison between different array analyses. All samples were tested in duplicate on a single plate and the average intensity was recorded for statistical analysis. To evaluate changes in neurofilament-heavy chain (NfH) following blast injury, a method similar to that presented by Petzold et al. was used. All samples were tested in duplicate on a single plate and the average intensity is recorded for statistical analysis.

Results

We have run all of the vitreous biomarkers from the experimental studies. We added additional animals to this group due to some concerns with the accuracy of a small subset of the data. The new studies have been completed and the assays run. Analysis on the arrays is underway. We will incorporate the data into our existing statistical matrix and complete the analysis this next quart. Even without this supplemental data, we have found significant decreases in contralateral LIX, and TNF-α up to four weeks post-injury (**Figure 9**) and a significant increase in ipsilateral LIX and TNF-α compared to the contralateral eye, but only at four weeks. No significant changes were found in VEGF. LIX (CXCL5/LPS-induced chemokine) is involved in neutrophil recruitment to the corneal stroma. Corneal damage has been seen in the contralateral eye at 1 week, but it resolves at 2 weeks. This could explain the decrease in LIX at 4 weeks compared to 1 week post-blast in the contralateral eye. Corneal injury in the ipsilateral eye appears at 2 weeks in the stroma and at 6 weeks in the epithelial layer. For this reason, it makes sense that LIX is significantly higher in the ipsilateral eye at 4 weeks compared to the contralateral eye. TNF-α is a proinflammatory cytokine that is associated with many diseases. It is not clear whether injury to the cornea initiated the increase of this protein, or injury elsewhere to the eye. The response is very similar to LIX, so we believe it is related to the corneal damage.

Neurofilament Heavy Chain (NfH) significantly increased immediately after injury and was maintained up to 4 weeks after injury and began to decline at 8 weeks (**Figure 10**). NfH is believed to be a marker of retinal degeneration. This implies that the retina from the blast has sustained breakdown until after 4 weeks. Behaviorally, we see immediate and sustained vision deficits which correlate with these biomarker findings. Vision deficits seen after 4 weeks could be due to the manifestation of corneal injury by that time point.

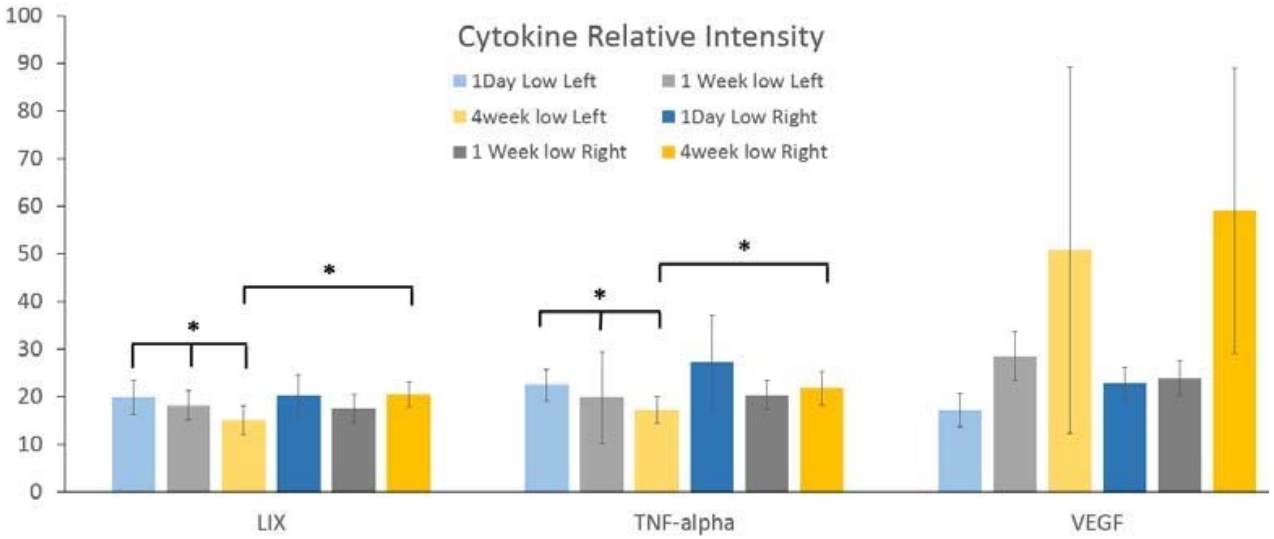


Figure 9. Results of protein biomarker analysis 1 day, 1 week, and 4 weeks after blast exposure. LIX and TNF-alpha significantly decreased in the contralateral blast eye, but were significantly increased in the ipsilateral eye at 4 weeks. This is likely related to the temporal response of the corneal injury. VEGF showed some trends, but these are not significant at this time.

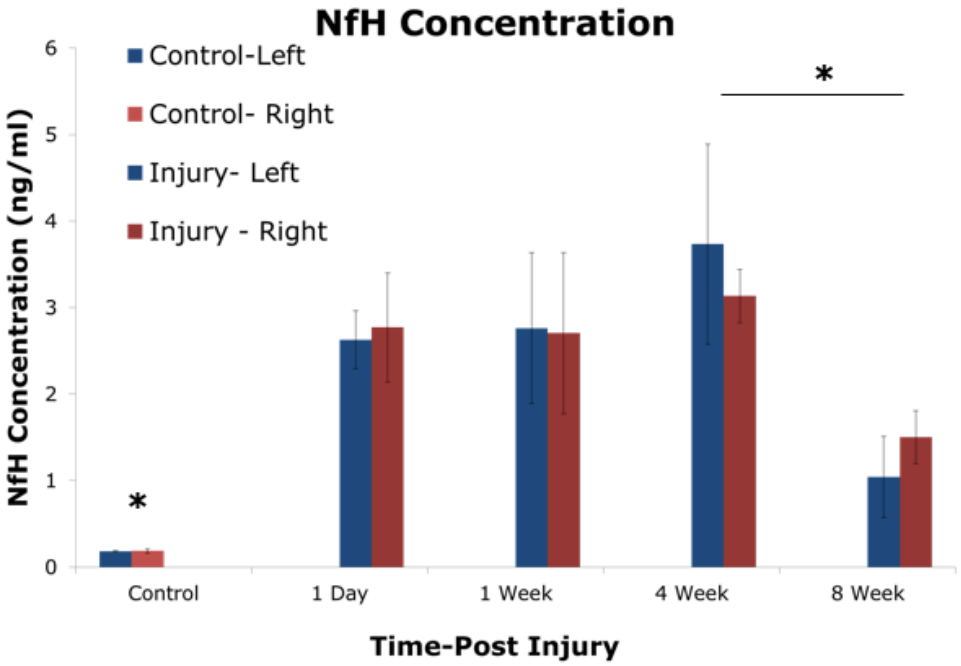


Figure 10. Neurofilament heavy chain (NfH) significantly increased in both the left and right eyes following blast exposure and was sustained for 4 weeks post injury.

Progress and Accomplishments

The vitreous findings were presented in a poster titled “Temporal Changes in Vitreous Inflammatory Cytokines and Neurofilament Heavy Chain Following Ocular Blast Trauma” at the February 016 ARVO conference.

The final set of vitreous samples has been processed. We are currently working to integrate our data sets, rerun our statistical evaluation, and prepare this data for publication.

KEY RESEARCH ACCOMPLISHMENTS:

- Identified immediate decrease in vision following a low-level blast exposure that remains steady until 8 weeks post injury. This was significantly different than control animals which actually improved with time.
- Characterized significant temporal corneal changes following blast exposure. In eyes not directly exposure to a pressure wave, these changes appear to occur early on, but resolve. In eyes directly exposed to a pressure wave, the stroma thickens after 2 weeks, then the epithelial layer thickens at 5 weeks. Eventual corneal scarring occurs in many of the animals. The initial identification of corneal thickening provides a window of opportunity for drug treatment that may prevent eventual scarring.
- No significant effects of blast exposure on retinal thickness were identified. Injury to the retinal layers could require a longer duration of evaluation (i.e., greater than 8 weeks).
- Designed a way to measure intraocular pressure during blast exposure. Found that peak pressure from a tube sensor is adequate to estimate direct blast exposure to the eye, but does not accurately capture the rate of the blast wave, nor any of the load metrics for an indirectly exposed eye.
- There is a significant increase in LIX and TNF-a at time points correlating to structural changes in the cornea. These protein changes may be influential in identifying appropriate drug treatment targets.
- A significant increase in NfH immediately post-blast correlates well with the findings of immediate visual acuity loss post-blast. This suggests that retinal damage may be responsible for immediate changes in vision, but subsequent vision loss may be due to both retinal and corneal injury. Future studies should investigate the contribution of each of these injuries to vision degradation.
- Completed the retrospective analysis and identified a cohort for prospective study evaluation. These studies are in progress.

CONCLUSION:

The successful completion of the studies proposed in this 4 year project will form the basis for understanding the temporal and chemical progression of visual system injury following blast exposure. In the first year, all the infrastructure and product development was completed to successfully achieve the stated goals of the study. In Year 2, the bulk of the experimental work was performed. Several modifications to the blast device were made. In Year 3, the bulk of animal studies were completed and resulted in some remarkable findings regarding the time course of corneal

injury and vision following a blast exposure. During Year 4, the retrospective human study moved to the data analysis phase, the prospective study began, and the last experimental animal studies were completed. The results from the animal studies will be critical to the development of treatment strategies to prevent vision loss in military personnel following blast exposure. We hope to link the findings of the animal studies to the prospective evaluations in the granted no-cost extension period. Dissemination of findings is also a major objective of the no-cost extension period.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Abstract & Presentation

Coats B, Shedd DF, Jones J. Temporal Changes in Vitreous Inflammatory Cytokines and Neurofilament Heavy Chain Following Ocular Blast Trauma. Association for Research in Vision and Opthalmology Annual Meeting 2016. Seattle, WA. May 2016.

Shedd DF, Jones J, Coats B. Ocular Injury following Low Level Blast Exposure in Rats. Association for Research in Vision and Ophthalmology Annual Meeting 2015. Denver, CO. May 2015.

Jones J, Shedd DF., Coats B. The Temporal Change in Protein Biomarkers in the Vitreous Humor following Blast Trauma. Summer Biomechanics, Bioengineering and BIotransport Conference 2015. Park City, UT. June 2015

Shedd DF, Jones J, Zaugg B., Coats B. Cornea Damage Progression following Blast Exposure. Summer Biomechanics, Bioengineering and BIotransport Conference 2015. Park City, UT. June 2015

Shedd DF and Coats B. Temporary visual dysfunction following low-level blast exposure. 7th World Congress of Biomechanics. Boston, MA July 2014

Book Chapter

Coats B. and Shedd DF. Biomechanics of Eye Injury in the Military. In A. Gefen & Y. Epstein (Eds) *Mechanobiology and Mechanophysiology of Military-Related Injuries.* New York: Springer (*in press 2016)*

INVENTIONS, PATENTS AND LICENSES:

Nothing to report.

REPORTABLE OUTCOMES:

- Silencer and dump tank developed for 12” diameter shock tube. Results in minimal change to the resulting pressure profile and results in a 15% reduction in decibel level.
- Designed a clamping system to pressurize shock tubes to high pressures and reduce early membrane failure.
- Developed semi-automated image processing tools for analyzing the thickness of the retina and cornea from OCT data.
- Developed automated image processing tools for analyzing cytokine biomarkers and NfH protein assays.
- Identified the time course of corneal injury following blast exposure.
- Identified initial biomarkers for corneal scarring following blast exposure.
- Identified the time course of cytokine and neurofilament heavy chain changes in the vitreous following blast exposure which help explain the mechanisms of vision loss.
- Developed novel method for measuring intraocular pressure during blast exposure.

OTHER ACHIEVEMENTS:

Nothing to report.

REFERENCES:

Petzold et al. A specific ELISA for measuring neurofilament heavy chain phosphoforms. *Journal of Immunological Methods.* 278, 179-190 (2003).

APPENDICES:

Appendix A: Prospective Recruitment Flyer
Appendix B: 2014 World Congress of Biomechanics Abstract
Appendix C: 2014 World Congress of Biomechanics Poster
Appendix D: Research Highlight of this proposal included in the FY14 Report to the executive Agent for Prevention, Mitigation, and Treatment of Blast Injuries.
Appendix E: 2015 ARVO abstract – Podium presentation
Appendix F: 2015 Summer Biomechanics, Bioengineering and Biotransport Conference Abstract (PhD Competition Finalist)
Appendix G: 2015 Summer Biomechanics, Bioengineering and Biotransport Conference Abstract (2nd place winner in the undergraduate research competition)
Appendix H: 2016 ARVO Poster
Appendix I: Brittany Coats (PI) CV
Appendix J: Quad Chart

Appendix A: Prospective Recruitment Flyer

VISION CHANGES FOLLOWING BLAST EXPOSURE

PAID VOLUNTEERS NEEDED

FREE VISION EXAMS

NON-INVASIVE

HELP US IDENTIFY BLAST MECHANICS LEADING TO AN INCREASED RISK OF DECREASED VISION

Participants Needed for Research Study on Vision Changes Following Blast Exposure

The Ocular Biomechanics Lab at the University of Utah, in collaboration with the Salt Lake City VA Hospital, are investigating changes in vision following direct or indirect exposure to a blast. We are looking for volunteers to participate in a 1 to 2 year study evaluating possible changes in vision following blast exposure. Participants will be interviewed, fill out questionnaires, and receive eye examinations every four months. Participants will receive a $50 gift card for every eye examination study visit.

If you are interested in learning more and/or participating in the study, please visit the following URL:

http://j.mp/29j7kAx

QUESTIONS?

Feel free to contact the Principal Investigator:

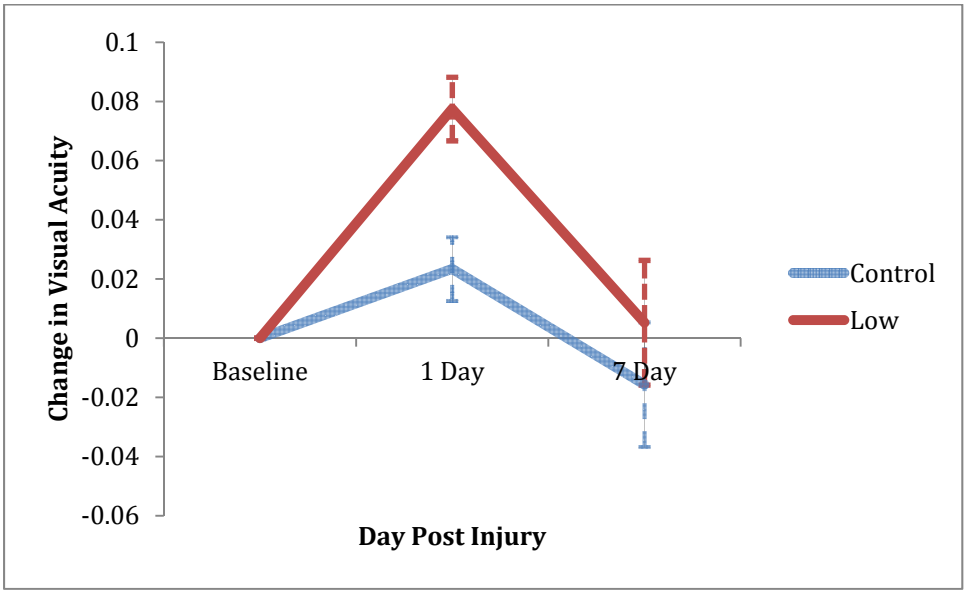
Brittany Coats, PhD
Mechanical Engineering
University of Utah
brittany.coats@utah.edu
801-585-0586

USAMRAA
UNITED STATES ARMY
Medical Research Acquisition Activity

Project funded through the generous support of USAMRAA grant#W81XWH-12-1-0243

Title: Temporary Visual Dysfunction following Low Level Blast Exposure

Blast exposure is a leading cause of eye injury for the US Army. Open globe ocular trauma, including shrapnel or debris to the eye, is easily identified and rapidly treated. Closed globe trauma may not be detected right away, and little is known about the time course of visual dysfunction following blast exposure. To better understand the mechanisms behind blast induced vision loss, we have developed a rodent model to characterize the time-dependent changes in visual acuity after blast exposure. To assess visual acuity in rodents, a custom vision behavioral device was built to measure the threshold for the natural optokinetic nystagmus reflex. The test animal is placed in the center of the device and a cylindrical sine wave grating is displayed on four surrounding computer monitors. The grating rotates around the animal, which causes the animal to reflexively track the grating motion with head movements. The level of grating contrast at which the direction of drift is correctly tracked by the animal represents the level of functional visual acuity. An increase in visual acuity indicates a decrease in vision functionality. For the present study, anesthetized Long-Evans rats were exposed to 230 kPa pressure waves using a compressed-air shock tube. Control animals were anesthetized and placed in the shock tube, but no pressure wave was activated. Visual acuity was assessed three times in each animal at three time points: before blast exposure, one day after exposure, and one week after exposure. Relative to baseline measurements, animals exposed to the blast pressure wave had a significant increase from visual acuity one day after the blast and then returned to pre-injury levels one week after the blast. No increase was found in control animals. This suggests that a low level blast may cause temporary visual dysfunction, but it is not sufficient to cause long-term injury. Future studies will investigate visual functionality at more severe levels of blast exposure and for later time periods after blast exposure.



Temporary Visual Dysfunction following Low Level Blast Exposure

Daniel F. Shedd and Brittany Coats, PhD
Mechanical Engineering, University of Utah, Salt Lake City, UT USA

THE UNIVERSITY OF UTAH

PEDIATRIC HEAD TRAUMA
Injury Biomechanics Lab

Introduction

Blast exposure is a leading cause of eye injury for the US Army [1]. Typically, ocular injury occurs from explosive shrapnel and debris, but recently many soldiers have developed vision deficits 6-12 months following a blast exposure without any signs of injury [2]. Closed globe trauma may not be detected right away, and little is known about the time course of visual dysfunction following blast exposure. To better understand the mechanisms behind blast induced vision loss, we developed a rodent model to characterize the time-dependent changes in visual acuity after blast exposure using behavioral vision testing and optical coherence tomography (OCT).

Methods

Anesthetized Long-Evans rats (300-350g, n=12) were exposed to 230 kPa pressure waves using a compressed-air shock tube (Fig. 1). Control animals (n=12) were anesthetized and placed in the shock tube, but no pressure wave was activated. Animals were euthanized at 1 day, 1 week, 4 weeks, or 8 weeks post-blast.

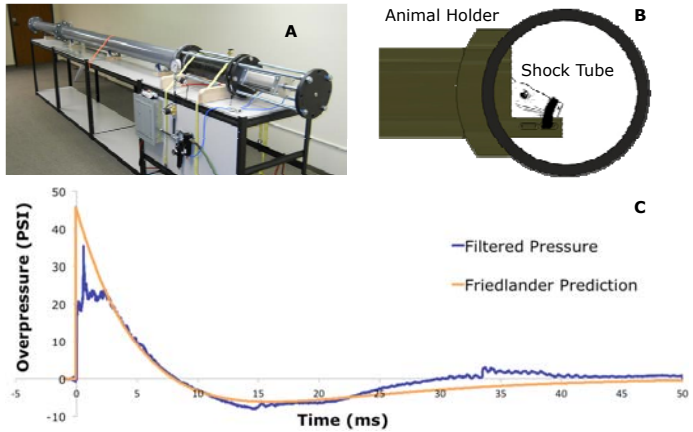


Fig. 1. (A) The 6″ experimental shock tube was triggered via rupturing BoPET membranes and instrumented with 1 MS/s pressure sensors (PCB 113B26) along the length of the driven section. **(B)** Animal placement within shock tube. **(C)** Representative filtered pressure profile used to apply blast insult. Comparison to ideal Friedlander waveform shown. R^2 = .92

A custom vision behavior device (Fig. 2) was built to measure the visual acuity threshold using the optokinetic nystagmus reflex. Test animals were placed in the center of the device and a cylindrical sine wave grating was displayed on four surrounding computer monitors. The grating rotated around the animal, which caused the animal to reflexively track the grating motion. The grating contrast at which the direction of drift was tracked by the animal represented the level of functional visual acuity (Fig. 3). Visual acuity was assessed three times in each animal at up to eight time points. A two-tailed matched-pair test with p=.05 was used to find significant vision changes. OCT imaging (Fig. 4) was performed using Bioptigen Envisu™ R2200 OCT scanner with an ultra-high resolution (UHR) light source and a rat retina lens. The scan settings were: 1000 A-scans per B-Scan, 100 B-scans over a field of view of 2.6 mm by 2.6 mm. Images were processed and analyzed using MATLAB [3] to find total retinal thickness and RPE thickness.

More information about the Utah Laboratory of Pediatric Injury Biomechanics is available at our lab website: pedtrauma.mech.utah.edu

Methods

Figure 2. Visual acuity behavior test device

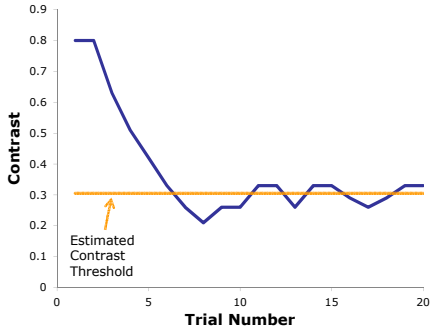


Fig. 3. Representative plot identifying visual acuity in a rat following blast exposure.

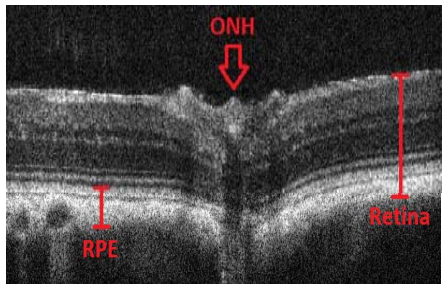


Fig. 4. Representative B-scan centered at optic nerve head (ONH). Total retinal thickness and RPE thickness were extracted across retina width and averaged. For the purposes of analysis, RPE included the photoreceptor inner/outer segments.

References

[1] DeFraites, R., *et al.*, Medical Surveillance Monthly Report, May 2011: 2-6. 2011.
[2] Cockerham, G., *et al.* Eye and visual function in traumatic brain injury. *Journal of Rehabilitation Research and Development* 46, 811-818. 2000.
[3] MATLAB and Image Processing Toolbox Release 2012b, The MathWorks, Inc., Natick, MA.

Results

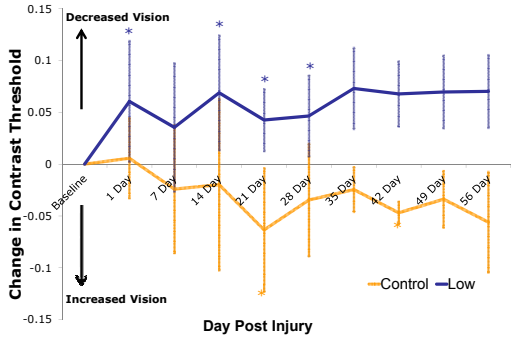


Fig. 5. Change in contrast threshold from baseline over time for control and low-level blast-exposed animals. * p<.05

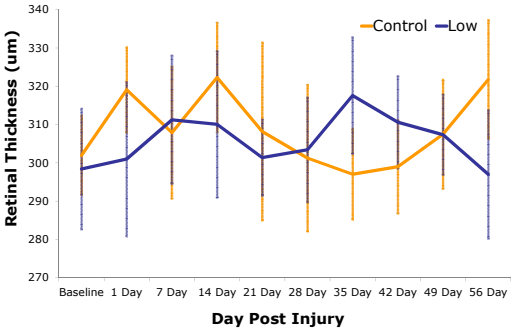


Fig. 6. Thickness of retinal layer over time, representing blast animals (n=3) and control animals (n=3). All data was gathered from eyes ipsilateral to blast insult. No significant trends were found.

Conclusions

- **Blast-exposed animals exhibited decreased visual acuity at one day, two week, three week, and four week time points as measured by behavior testing.**
- **Control animals exhibited unchanged visual ability, with the exception of increased visual ability at three week and six week time points. This may be due to increased comfort with the behavior system.**
- **Retinal thickness did not significantly change in either group at any time point.**

Acknowledgements

We would like to thank *USAMRMC #W81XWH-12-1-0243* for support of this project .

Contact Information

Daniel F. Shedd
Email: d.shedd@utah.edu
Brittany Coats, PhD
Email: brittany.coats@utah.edu

APPENDIX C - Poster for 7th World Congress of Biomechanics

APPENDIX D - Research Highlight included in the FY14 Report to the executive Agent for Prevention, Mitigation, and Treatment of Blast Injuries.

DATA CALL SUMMARY
Please review and approve

Temporal Progression of Visual Injury from Blast Exposure

Dr. Brittany Coats from the University of Utah's Department of Mechanical Engineering is conducting research funded by a U.S. Army Medical Research Acquisition Activity (USAMRAA) grant W81XWH1210243 to investigate the temporal progression of eye injury from blast exposure and identify early predictors of visual dysfunction. Although ocular trauma is not uncommon in modern day military conflicts, closed globe injury may not be detected immediately, and can result in sequelae that lead to visual dysfunction months after the blast exposure. Furthermore, the progression of closed globe eye injury and visual degradation following blast exposure has not been well characterized, and it is unknown if there are early indicators that denote an increased risk for developing visual dysfunction following blast exposure. Two studies comprise Coats' current work on the progression of visual system injury: (1) a retrospective and prospective analysis of Service Members exposed to a blast, and (2) an experimental study using a rat model to evaluate retinal and corneal damage as well as vitreous protein expression. The first study is ongoing. The results of the second study using the rat model indicate that there is an immediate decrease in vision following a low-level blast exposure that remains steady until 8 weeks post injury. Corneal damage resulted from blast pressure alone, but wasn't identifiable until 3 weeks after the blast. The work from this project has resulted in a collaboration with Dr. Barbara Wirostko, CSO of Jade Therapeutics, Inc., who is also funded by the USAMRAA to develop biodegradable biofilms that can be placed in the eye for drug delivery. It is Coats' and Wirostko's hope that Jade's novel crosslinked hyaluronic acid polymer can prevent or treat corneal damage resulting from blast exposure. The successful completion of these studies will expand our understanding of the time-dependent response of the visual system to blast, enhance current diagnostic capabilities, and lead to the development of time-dependent treatment strategies to mitigate the loss of vision in military personnel.

Please complete below so that the summary can be finalized

Performing organizations:	University of Utah, Department of Mechanical Engineering
Sponsoring organizations:	USAMRAA grant W81XWH1210243
Approved by:	Brittany Coats
Date:	March 26, 2015
Comments:	

CONTROL ID: 2178343
SUBMISSION ROLE: Abstract Submission

AUTHORS

AUTHORS (LAST NAME, FIRST NAME): Shedd, Daniel[1]; Coats, Brittany[1]
INSTITUTIONS (ALL):
1. Mechanical Engineering, University of Utah, Salt Lake City, UT, United States.
Commercial Relationships Disclosure (Abstract): Daniel Shedd: Commercial Relationship: Code N (No Commercial Relationship) | Brittany Coats: Commercial Relationship: Code N (No Commercial Relationship)
Study Group: Developmental Head Injury Biomechanics Laboratory

ABSTRACT

TITLE: Visual Dysfunction Following Low Level Blast Exposure in Rats

ABSTRACT BODY:
Purpose: Blast exposure is a leading cause of eye injury for the US Army. Closed globe trauma may not be detected right away, and little is known about the time course of visual dysfunction following blast exposure. To better understand the mechanisms behind blast induced vision loss, a rodent model was developed and used to characterize the time-dependent changes in visual acuity after blast exposure using behavioral vision testing and optical coherence tomography (OCT).
Methods: Anesthetized Long-Evans rats (300-350g, n=26) were exposed to 230 kPa pressure waves using a 6 inch diameter compressed-air blast tube. Animals were evaluated at 1 day post-blast and weekly up to 8 weeks post-blast. A custom vision behavior device was built to measure the visual acuity threshold using the optokinetic nystagmus reflex. Test animals were placed in the center of the device and a cylindrical sine wave grating was displayed on four surrounding computer monitors. The grating rotated around the animal, which caused the animal to reflexively track the grating motion. The contrast of the grating at which the direction of drift was tracked by the animal represented the level of functional visual acuity. Three trials were completed for each animal at each time point. A two-tailed matched-pair test with p=.05 was used to find significant vision changes. OCT imaging (Bioptigen Envisu™ R2200) with an ultra-high resolution (UHR) light source was used to identify changes in retinal thickness in 8 regions around the optic nerve.
Results: There was a significant reduction in visual acuity in all rats 1 day after blast exposure (Figure 1). This reduction was sustained for the duration of the study. The visual acuity in control animals (n=26) increased after day one and remained stable up to 8 weeks. Retinal thickness was normalized to baseline values and compared with controls. Several regions of the posterior retina thickened slightly (~8%) at week 2, but was resolved by week 8.
Conclusions: Low level blast exposure results in an acute decrease in visual function that was sustained up to 8 weeks. The blast also resulted in delayed changes in retinal thickness which resolved over a month. Additional studies are underway to evaluate the electrical physiology of the retina to support theh findings of decreased functional visual acuity.

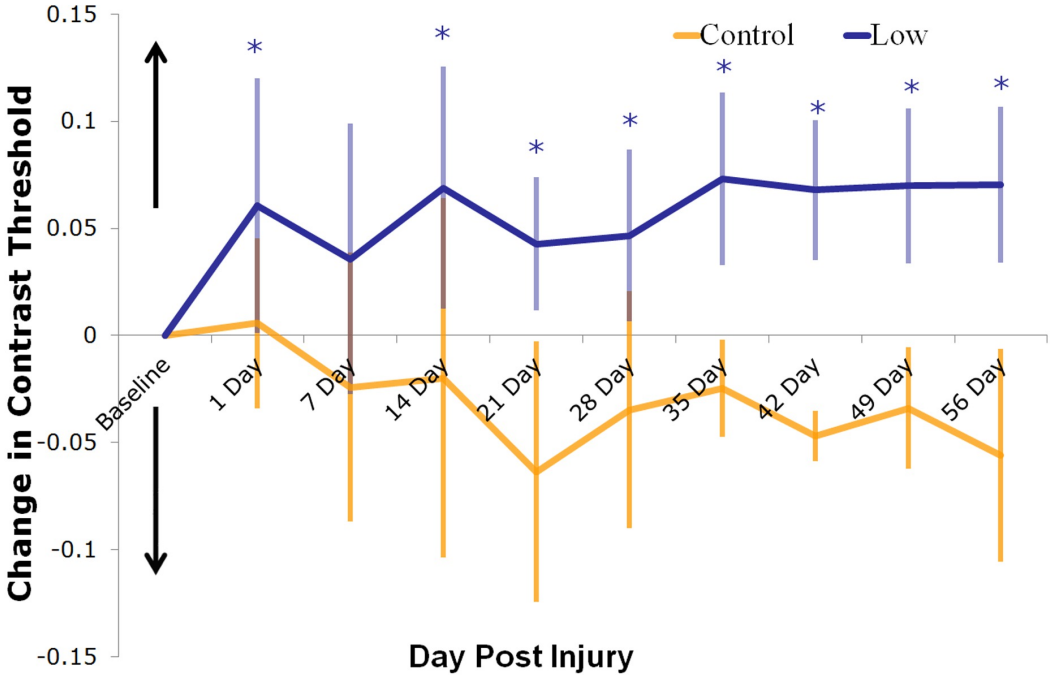


Figure 1. Change in contrast threshold from baseline over time for control and low-level blast exposed animals. *p<0.05.

DETAILS

PRESENTATION TYPE: #1 Paper, #2 Poster
CURRENT REVIEWING CODE: 3720 trauma: posterior segment, clinical - RE
CURRENT SECTION: Retina
KEYWORDS: 742 trauma, 688 retina, 730 temporal vision.
Clinical Trial Registration (Abstract): No
Other Registry Site (Abstract):
Registration Number (Abstract):
Date Trial was Registered (MM/DD/YYYY) (Abstract):
Date Trial Began (MM/DD/YYYY) (Abstract):
Grant Support (Abstract): Yes
Support Detail (Abstract): USAMRMC #W81XWH-12-1- 0243

TRAVEL GRANTS and AWARDS APPLICATIONS

AWARDS: ARVO and ARVO Foundation Travel Grants|ARVO 2015 Members-in-Training Outstanding Poster Award

SB^{3}C2015
Summer Biomechanics, Bioengineering and BIotransport Conference
June 17-20, 2015,Snowbird Resort, Utah, USA

CORNEA DAMAGE PROGRESSION FOLLOWING BLAST EXPOSURE

Daniel F. Shedd (1), Justin A. Jones (2), Brian Zaugg (3), Brittany Coats (1)

(1) Department of Mechanical Engineering
University of Utah
Salt Lake City, Utah, USA

(2) Department of Bioengineering
University of Utah
Salt Lake City, Utah, USA

(3) John A. Moran Eye Center
University of Utah
Salt Lake City, Utah, USA

INTRODUCTION

Blast exposure is a significant cause of injury for the US Army [1]. The time course of eye injury subsequent to exposure is not well understood, especially for cases of closed globe trauma. Several studies have investigated visual impairment in soldiers with traumatic brain injury from blast exposure. They report ~75% of soldiers with traumatic brain injury also have visual dysfunctions [2,3]. One study in particular performed complete ocular exams on all soldiers with a history of traumatic brain injury from blast exposure and found retinal injuries in several military personnel that were unaware they had any ocular or visual problems [4].

To investigate the time course of blast induced vision loss, we developed a high-pressure blast injury model in the rodent. Our objective in this study was to evaluate the long-term (8 week) time course of corneal injury following blast exposure.

METHODS

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Utah. Long-Evans rats (300-350g, n=38) were anesthetized using IP injection of a ketamine-dexmedetomidine mix and exposed to blast waves (peak overpressure = 230 kPa) using a compressed air driven shock tube (Fig. 1). Firing of the shock tube was controlled by material failure of a biaxially oriented polyethylene (BoPET) membrane (.01" thickness), which occurred at a driver section overpressure of 650-750 kPa. A representative blast wave generated at the location of the animal is shown in Fig. 2.

The animals were placed inside of the tube using a 3D-printed mount exposing the head and eyes to a side-on blast insult, while protecting the body and lungs from the injury. Additional protection of the body was achieved by wrapping the anesthetized animal in a Kevlar shroud. The right eye of the animal was always ipsilateral to the oncoming pressure wave throughout the study. A subset of blast studies were videotaped using a Phantom high speed camera at 5000 fps to assess head motion induced by the blast wave. The video data was also used to ensure that the rupturing membrane did not generate any shrapnel, as this could cause additional injuries to the animal.

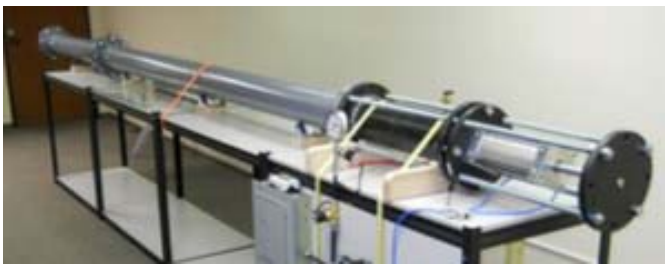


FIGURE 1. 6-IN INTERNAL DIAMETER SHOCK TUBE USED TO GENERATE FRIEDLANDER SHAPED BLAST WAVES.

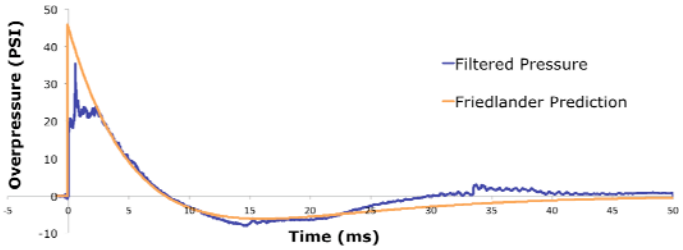


FIGURE 2. BLAST WAVE MEASURED 1-IN IN FRONT OF ANIMAL LOCATION COMPARED TO PREDICTION OF BLAST FROM FRIEDLANDER WAVE EQUATION

At time points 1 day before blast, 1 day post blast, and every week after blast up to 8 weeks, corneal imaging of both left and right eyes was performed using a Bioptigen Envisu R2200™ OCT scanner with an ultra-high resolution light source (Telecentric lens, 4.0 x 4.0 FOV, 100 B scans, 1000 A scans).

Gross ocular examinations were performed at each time point to determine the presence of any easily identifiable injury or corneal defects. When possible, an ophthalmologist (BZ) performed ophthalmic exams of the eyes. Fluorescein staining was used to aid in visualization of superficial corneal epithelium injuries.

Overall corneal thickness was measured regionally using a MATLAB code created using the Image Processing Toolbox. Stromal and epithelium thickness were measured manually using InVivo Vue software (Bioptigen, North Carolina).

A Dunnett's test was used to compare the corneal, stromal, and epithelial thicknesses at every time point to the baseline (pre-injury) measurement (JMP, SAS Institute, North Carolina). A p-value < 0.5 was considered significant.

RESULTS

The superficial epithelium layer and the underlying stroma layer could be clearly resolved in all images (Figure 3A).

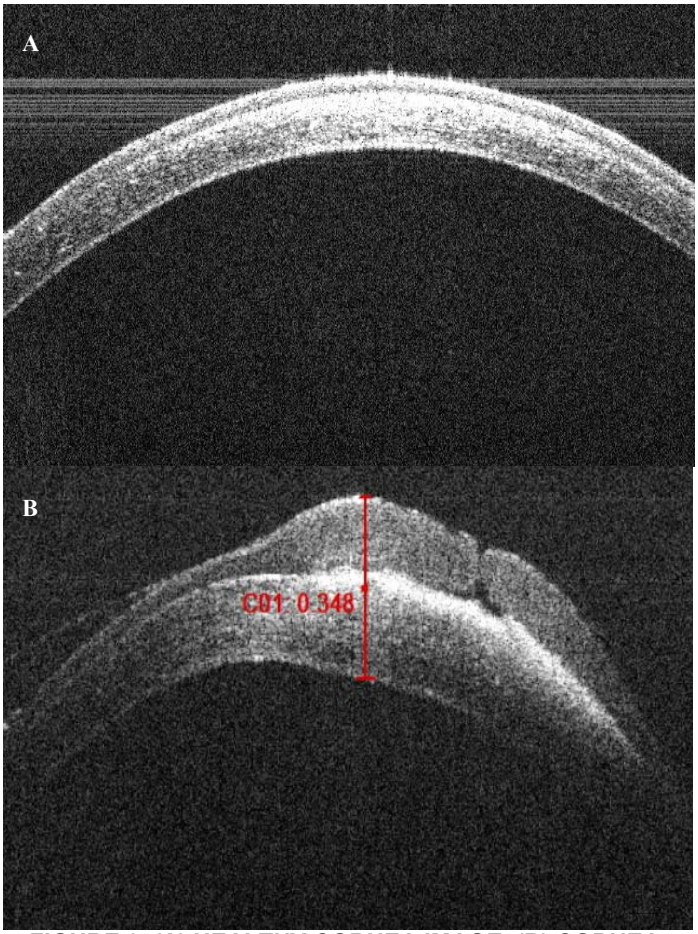


FIGURE 3. (A) HEALTHY CORNEA IMAGE. (B) CORNEA EXHIBITING THICKENING OF EPITHELIUM INDICATIVE OF INFLAMMATION. THE CORNEAL STROMA IS ALSO SLIGHTLY ENLARGED, AND THE BORDER BETWEEN LAYERS IS UNHEALTHY.

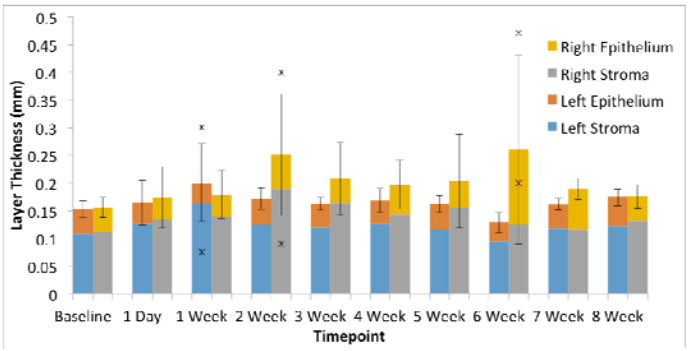


FIGURE 4. AVERAGE THICKNESS FOR TOTAL CORNEA AS WELL AS STROMA AND EPITHELIUM. STARRED COLUMS INDICATE STATISTICAL SIGNIFICANCE COMPARED BASELINE (PRE-INJURY).

In the eye contralateral to the blast (left), significant changes in overall corneal thickness and stromal thickness (p=.0105) occurred at one week (Figure 4). These changes resolved by two weeks after blast. The eye ipsilateral to the blast (right) had significant increases in corneal thickness at two (p=.0066) and six weeks (p=.0167). At two weeks, the increased thickness came from welling of the stroma, while at six weeks the thickening was due to epithelial changes. These data points correlated with the appearance of visible gross injury to the cornea at 3-4 weeks followed by scarring at 6-8 weeks, as well as neovascularization.

DISCUSSION

The blast exposure appears to have caused a structural injury in the stroma of the right eye. This defect develops into a measurable change in thickness of the stroma which is at first not visible on the surface of the eye. This injury may be caused by a disorganization or disruption of collagen plates that make up the corneal stroma. The stroma eventually heals, returning to pre-injury thickness, but leaves behind unhealthy epithelial tissue, which presents as a superficial hemorrhage and eventual scarring. Interestingly, there was transient thickening of the left cornea which was contralateral to the blast. The thickening resolved quickly and did not result in gross indications of injury.

These data show that blast exposure can cause delayed expression of injury to the cornea. The injury is not limited to the ipsilateral side of blast exposure, but may also be present contralateral to a lateral blast. These injuries may also be measurable by other types of collected data, such as vision behavioral studies or protein expression, which are not presented in this work. A better understanding of these injuries and their time course will aid in the detection and treatment in blast-exposed individuals.

ACKNOWLEDGEMENTS

We would like to thank USAMRAA #W81XWH-12-1-0243 for support of this project. We'd also like to thank Krishna Womack for her assistance with data analysis.

REFERENCES

[1] Ari AB., *Optometry*, 77:329-339, 2006.
[2] Kapoor, N. et al., *Neurology* 4:271-280, 2002.
[3] Brahm, K. et al., *Optometry and Vision Science* 86:817-825, 2009.
[4] Cockerham, G, *et al. J Rehabil Res Dev* 46: 811-818, 2009.

SB^{3}C2015
Summer Biomechanics, Bioengineering and BIotransport Conference
June 17-20, 2015,Snowbird Resort, Utah, USA

The Temporal Change in Protein Biomarkers in the Vitreous Humor following Blast Trauma

Justin A. Jones (1), Daniel F. Shedd (2), Brittany Coats (2)

(1) Department of Bioengineering
University of Utah
Salt Lake City, Utah, United States

(2) Department Mechanical Engineering
University of Utah
Salt Lake City, Utah, United States

INTRODUCTION

Ocular injuries due to blast exposure have increased in occurrence over the last several decades. Between 1983 and 2002, 36,110 bombings occurred in the United States, resulting in 5931 injuries [1]. The incidence of eye injury due to blast trauma with soldiers has increased from 9% to 13% since the Vietnam War [2]. Progression from ocular injury to ocular disease is not adequately characterized; and currently, indicators of injury progression to vision loss are largely unknown. Common closed globe injuries, including, retinal detachment, retinal tears, and optic nerve fiber degeneration [3], can elicit a cellular inflammatory response that releases proteins into the vitreous humor of the eye.

One prominent ocular protein biomarker is the neurofilament heavy chain (NfH). It is believed that NfH is released from degenerating retinal ganglion cells and their axons into the vitreous [4]. Other protein biomarkers of importance are cytokines. Cytokines are signaling proteins present in the inflammatory cascade. A particularly well known cytokine is vascular endothelial growth factor (VEGF) and has importance to pathologic angiogenesis [5]. Its subcomponents are said to be involved in endothelial cell migration, proliferation, survival and permeability and are typically present any time there is an inflammatory response [6].

The goal of this study was to discover if protein biomarkers known to reflect ocular injury can be used as reliable early identifiers of vision loss due to blast exposure.

METHODS

All testing procedures were approved by the University of Utah Institutional Animal Care Use Committee (IACUC). Male Long Evans rats (n = 24; 300-350g) were placed in a 6 meter long by 15.24 cm internal diameter blast tube, and exposed to a 30 psi overpressure blast with a 7 msec duration (Figure 1). Experimental animals were separated into three survival time groups: 1 day, 1 week and 4 week. Before the blast exposure was performed, each animal was weighed and anesthetized using a mix of ketamine and dexmedetomidine with a dosage of 65mg/kg and 0.14mg/kg, respectively. A visual eye examination was then performed by an ophthalmologist. The animals were exposed to a blast perpendicular to the sagittal plane from right to left. After the blast, the animal was removed and another visual eye examination was performed.

After the respective survival time was reached, the animals were sacrificed by formalin perfusion fixation through the heart using standard practices [7]. The whole globe eyes and brains were harvested at necropsy. The eyes were then eviscerated and the vitreous and lens removed. The vitreous and lens were separated by placing them into a filter centrifuge tube and spun down (10k rpm, 10 min). The separated vitreous (approx. 50 µL) was diluted with phosphate buffer saline (PBS) until a total volume of 150µL was reached. The sample was then separated into three equal tubes.

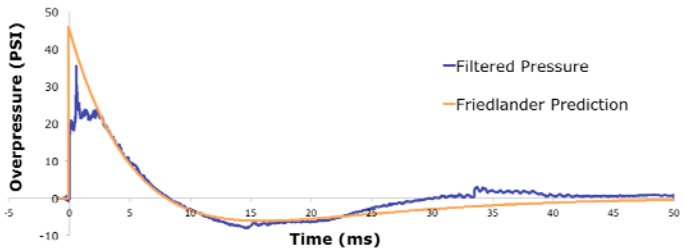


FIGURE 1. PRESSURE-TIME HISTORY AT LOCATION OF ANIMAL PLACEMENT WITHIN BLAST TUBE

To analyze the NfH content, an ELISA protocol was used according to an ELISA technique previously developed by Petzold et al [8]. The microtitre plates were coated overnight at 4°C with 100 µL of capture antibody, SMI35. The plates were then washed three times for 10 minutes using a barbitone buffer wash containing 0.1% BSA, and 0.05% Tween 20. After washing, 250µL of barbitone block with 1% BSA was added to each well and the plate was incubated at room temperature (RT) for 1 hour. After another wash cycle, 50µL of sample, standard, or negative control was added to each well of the plate in duplicate. After one hour incubation at RT the wash processes was repeated. After washing, 100µL of second antibody was added to each well of the plate and incubated for 1 hour at RT. Following a third wash cycle 100µL HRP-labeled swine anti-rabbit antibody was added to the plates and incubated for one hour at RT. After a final wash 100µL TMB substrate was added and incubated for 20 minutes in a dark room, the reaction was stopped by adding 50µL of 1 M HCL. The absorbance was then read using an ELISA plate reader at 450nm with 750nm reference wavelength.

The analysis of inflammatory cytokines was performed using a commercially available kit (RayBio® Rat Cytokine Antibody Array G). These kits tested for the 19 cytokines including VEGF, LIX and TNF-α. The methods to develop these kits were done according to the manufacturer's instructions and can be found on RayBio® Tech website. Once the glass chip was developed, the intensities were read using a GENEPIX™ 4000A microarray scanner at an excitation frequency of 532nm.

At this point in time, sample size is small (n=4 per group), so 5 one-way ANOVAs were performed. Two assessed significant changes across time points within each eye side, and three assessed significant differences between the right and left eye at each time point. Collection of control data is ongoing and is not included in this abstract. All analyses were performed using JMP® software with a p-value < 0.05 considered significant.

RESULTS

Both the left and right eyes showed a general increase in NfH concentration, but this increase was only significant in the right eye between 1 day and 4 weeks (p=0.044, Figure 2). There were no significant differences between NfH concentration of the ipsilateral (0.126±0.02 ng/ml) and contralateral (0.137±0.05 ng/ml) eyes at the 4 week time point.

Several cytokine proteins in the eye contralateral to the injury significantly decreased over time post-injury (GM-CSF, IL-1β, IL-4, IL-10, LIX ,TNF-α), but remained relatively constant in the eyes ipsilateral to injury (Figure 3). At 4 weeks after the injury, LIX and TNF-α were significantly higher in the eye ipsilateral to the injury

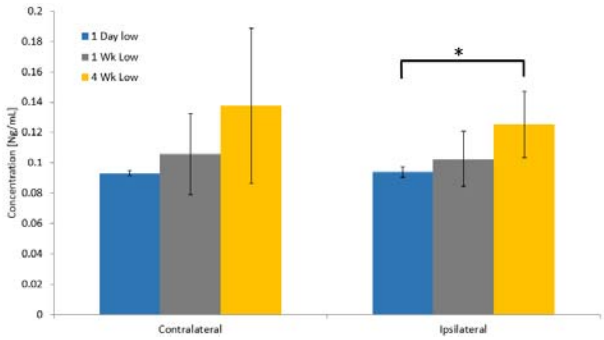


FIGURE 2. CHANGES IN NFH CONCENTRATION POST INJURY IN IPSILATERAL (RIGHT) AND CONTRALATERAL (LEFT) EYES. *p<0.05

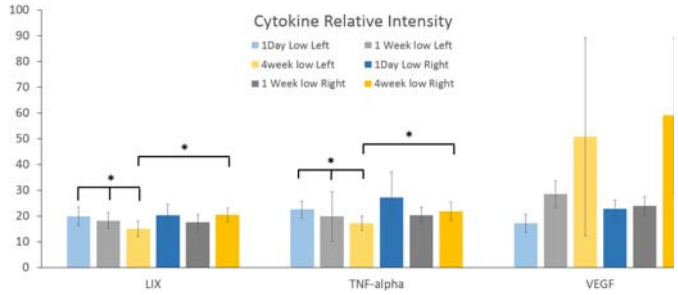


FIGURE 3. CYTOKINE RELATIVE INTENSITIES

compared to the contralateral eye (p = 0.015 and 0.043, respectively). No significance differences were seen in either of the eyes the remaining proteins of the cytokine array, including VEGF (shown above).

DISCUSSION

The significant decrease in NFH and cytokine proteins with time suggests that there was an acute (1 day) inflammatory response that occurred in both eyes, and only significantly decreased in the eye contralateral to the blast pressure. NfH is hypothesized to release from degenerating retinal ganglion cells and their axons. Previous research has shown that decreased axonal transport is preceded by cytoskeletal changes and degradation of NfH [4]. The sustained elevation of NfH in the ipsilateral eyes in this study suggest that cytoskeletal changes in the retina are ongoing at 4 weeks after injury. This long-term injury may lead to future vision degradation. Longer-term analysis, vision assessment and control group evaluation needs to be completed before making this assertion.

We found no significant change in VEGF in either eye. This was surprising as significant increases in VEGF have been reported in many ocular disorders including diabetic retinopathy, diffuse macular edema, retinal vein occlusion and retinal detachment [5]. Instead, we found several changes in interleukins, TNF-α, and LIX. The significance of these proteins in the blast injury response will be explored further do determine if they are merely a generic inflammatory response to the blast that is quickly resolved.

In summary, the blast pressure in this study appears to create some damage to the retina that is potentially recovered quickly in the contralateral eye, but not in the ipsilateral eye. Longer time points are currently being explored to determine the resolution of NfH to baseline levels post injury. Future work will include completion of the control groups as well as performing higher blast pressures to perhaps create higher levels of injury. We are also working to combine the findings of this study with changes in visual acuity and histology in the same animals.

ACKNOWLEDGEMENTS

We would like to thank USAMRAA #W81XWH-12-1-0243 for support of this project.

REFERENCES

[1] Kapur GB, et. al. *J Trauma,* 59:1436-44 2005.
[2] Weichel E., Colyer*, et al. Ophthalmology,* 115:2235-2245, 2008.
[3]Dougherty A., et al. *Brain Injury* 25:8-13, 2011.
[4] Balaratnasingam C. et al. *IOVS*, 49:986-999. 2008
[5]Cross M, et al. *TIBS,* 28:488-494, 2003.
[6]Miller J.*,* et al. *Am J Pathol* 145:574-584, 1994.
[7]Gage G. J, et al. *J. Vis. Exp.* (65), e3564, 2012
[8]Petzold A.*,* et al. *J Neural Transm* 116:1601-1606, 2009.

Temporal Changes in Vitreous Inflammatory Cytokines and Neurofilament Heavy Chain Following Ocular Blast Trauma

Brittany Coats PhD[1], Daniel Shedd[1], Justin Jones[2]

1 Department of Mechanical Engineering, University of Utah, Salt Lake City, UT

2 Department of Bioengineering, University of Utah, Salt Lake City, UT

THE UNIVERSITY OF UTAH

PEDIATRIC HEAD TRAUMA
Injury Biomechanics Lab

Appendix H: 2016 ARVO Poster

INTRODUCTION

The progression from ocular injury to ocular disease is not adequately characterized; and currently, indicators of injury progression to vision loss are largely unknown. Common closed globe injuries due to blast exposure include corneal swelling, neovascularization, retinal degeneration, and optic neuropathy. [1] Our current blast rodent model exhibits these injuries in a temporally multiplexed manner that may be exploited for protein biomarker detection and drug delivery.

The goal of this study was to measure proteomic changes to inflammatory cytokines and neurofilament heavy chain in the vitreous over 8 weeks following blast exposure and correlate the findings with the injury pathology time-course.

METHODS

The right side of anesthetized Long-Evans rats (300-350g, n=20) were exposed to 34 psi pressure waves using a compressed-air shock tube (Fig. 1). Control animals (n=12) were anesthetized and placed in the shock tube, but no pressure wave was activated. Animals were euthanized at 1 day, 1 week, 4 weeks, or 8 weeks post-blast.

After the respective survival time, the eyes were harvested and the vitreous humor removed. Neurofilament heavy chain (NfH), a protein resulting from degenerating retinal ganglion cells [2], was evaluated using an ELISA specifically developed for quantifying NfH concentration [3]. The absorbance was read using a Synergy HT Plate at 450 nm with a reference wavelength of 750 nm. Inflammatory cytokine changes were quantified using a commercially available kit (RayBio® Rat Cytokine Antibody Array G). A GenePix® 4000A micro array scanner and software were used to scan (532 nm) and analyze the arrays. The time-dependent response of all proteins, and comparison of experimental to control levels of concentration at each time point were evaluated with two one-way ANOVAs. A p-value < 0.05 was considered significant.

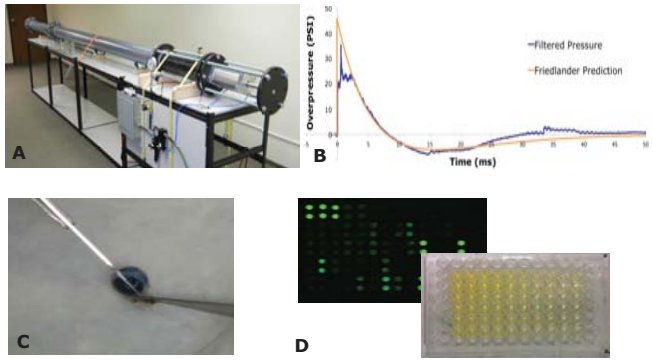


Fig. 1 (A) The 6" diameter experimental shock tube was triggered via rupturing BoPET membranes **(B)** Pressure of shock wave compared to Freelander wave form. **(C)** Vitreous removal by evisceration. **(D)** Biomarker assays resulting from the experiment.

RESULTS

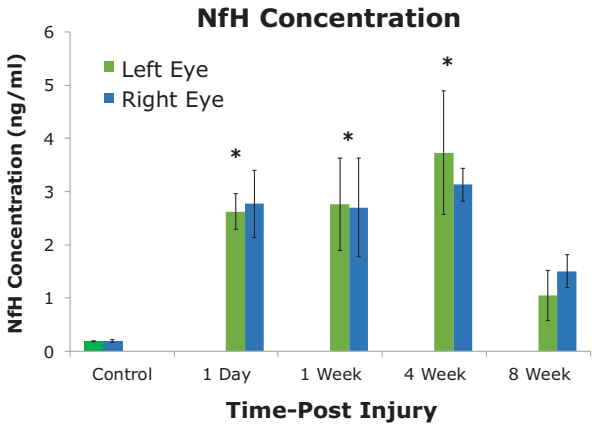


Fig. 2 NfH was significantly greater in injured animals compared to controls for all time points ($p < 0.05$) up to 4 weeks. At 8 weeks, NfH significantly decreased, approaching levels found in control animals.

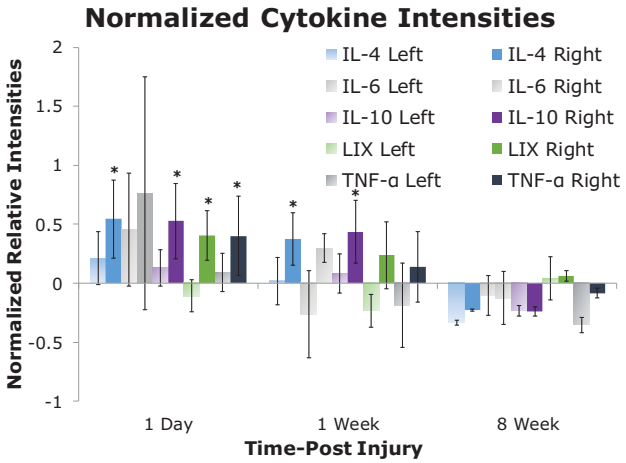


Fig. 3 Cytokines normalized by intensities in control animals. Both eyes had a general increased inflammatory response 1 day after injury, but returned to control levels by 8 weeks. Most significant changes were observed in the eye directly exposed to the blast (right eye).

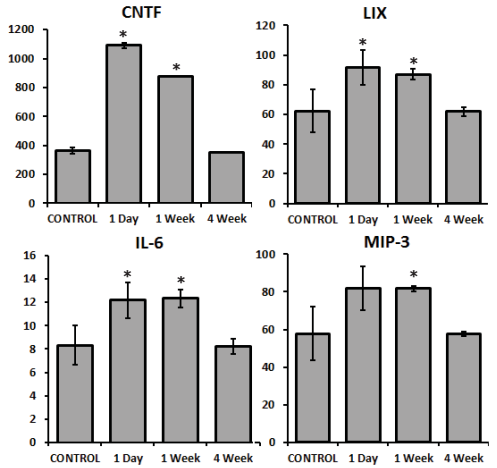


Fig. 4 Most cytokine changes returned to control values by 4 weeks, and were soon followed by structural changes observed on OCT (e.g., retinal thinning, corneal swelling/scarring).

KEY FINDINGS

- **A 34 psi blast is sufficient to elicit immediate proteomic responses in both eyes that typically resolve by 4 or 8 weeks.**
- **NfH concentration significantly increased immediately after injury and remained elevated until at least 4 weeks.**
- **Most inflammatory cytokines (IL-α, IL-β, IL-10, LIX, GMSF, Leptin, MIP-3, β-NGF) increased immediately after injury, and resolved by 4 or 8 weeks.**
- **The timing of these proteomic changes correspond to decreases in visual acuity in the animal model, and supersede structural changes to the retina (thinning) and cornea (swelling).**

CONCLUSION

The immediate vitreous proteomic changes prior to retinal and corneal pathology suggest that cytokines and NfH may be used as biomarkers for damage progression. Further, the profound inflammatory response identifies a potential avenue for exploring drug treatment to mitigate or reduce ocular damage from blast exposure.

ACKNOWLEDGEMENTS

We are very grateful to *USAMRMC #W81XWH-12-1-0243* for their financial support of this project.

CONTACT INFORMATION

Brittany Coats, PhD
brittany.coats@utah.edu
801-585-0586
Pedtrauma.mech.utah.edu

REFERENCES

[1] Dougherty A., et al., *Brain Injury* 25:8-13, 2011
[2] Balaratnasingam C. et al., *IOVS*, 49:986-999. 2008
[3] Petzold A., *J Neural Transm* 116:1601-1606, 2009

BRITTANY COATS

EDUCATION

University of Pennsylvania — ***Philadelphia, PA***
Doctorate of Philosophy, Bioengineering — May 2007
Advisor: Susan S. Margulies, Ph.D.
Dissertation: Mechanics of Head Impact in Infants

University of Utah — ***Salt Lake City, UT***
Bachelors of Science and Engineering, Mechanical Engineering — May 2000

RESEARCH EXPERIENCE

Associate Professor — ***University of Utah, 2016-Present***
Department of Mechanical Engineering — Salt Lake City, UT

Adjunct Affiliations — ***University of Utah, 2010-Present***
Department of Bioengineering — Salt Lake City, UT
Department of Pediatrics
Department of Ophthalmology and Visual Sciences

Assistant Professor — ***University of Utah, 2010-2016***
Department of Mechanical Engineering — Salt Lake City, UT

Post-doctoral Research Associate — ***University of Pennsylvania, 2007-2010***
•Department of Neurosurgery (2008-2010) — Philadelphia, PA
Traumatic brain injury following cyclic inertial head rotation in neonatal piglets.
•Department of Bioengineering (2007-2010)
Mechanics of ocular trauma in neonatal piglets following inertial, non-impact head rotation.

Graduate Research Assistant — ***University of Pennsylvania, 2000-2007***
Department of Bioengineering — Philadelphia, PA
Mechanics and skull tolerance of infants following impact events.

Research Technician — ***Becton Dickinson, 1998-2000***
Research and development of infusion therapy systems. — Sandy, UT

Undergraduate Research Assistant — ***University of Utah, 1997-1998***
Department of Bioengineering — Salt Lake City, UT
Cardiovascular monitoring during patient flight transport.

PENDING RESEARCH FUNDING

MTEC Consortium ***2016-2019***
Utah Vision Neuroprosthetic
Role: PI
Submitted: September 2016
Status: In review

National Science Foundation ***2017-2019***
Dynamics and Control of Magnetic Screws in Soft Tissue
Role: co-PI
Submitted: September 2016
Status: In review

AWARDED RESEARCH FUNDING

University of Utah Total:

National Institute of Justice ***2017-2019***
Skull fracture patterns from head impact in infants
Role: PI

National Science Foundation, SCH ***2016-2019***
SCH: INT: Reducing traumatic brain injury risk with smart collision detection and mitigation
Role: co-I

National Institutes of Health, R21EY025813-01A1 ***2016-2018***
Quantitative regional analysis of vitreoretinal adhesion with
age Total Award:
Role: PI

National Science Foundation ***2016***
SBIR Phase II: Biodegradable polymer film for sustained delivery of antibiotics to the surface of the eye
Total Award:
Role: co-PI

Department of Defense ***SBIR Phase II, 2015-2016***
Novel hyaluronic acid delivery polymer for repair of ocular
injuries Total Award:
Role: co-PI

Center for Child Injury Prevention ***2015-2016***
Pediatric brain injury assessment – YR 3
renewal Total Award:
Role: co-PI

National Institutes of Health ***NICHD, 2013-2017***
Repair of iatrogenic fetal membrane defects with an adhesive tissue scaffold

Total Award: Role:
co-PI

Early Career Development Award - Renewal ***PCMCF, 2013-2014***
Pediatric TBI from repetitive head rotation
Total Award:
Role: PI

Department of Defense Vision Research Program ***USAMRRA, 2012-2016***
Temporal progression of visual injury from blast
exposure Total Award:
Role: PI

University of Pennsylvania Vision Research Seed Grant ***UPENN, 2012-2013***
Decompression retinopathy and pediatric head trauma
Role: co-PI

Knights Templar Eye Foundation ***KTEF, 2012-2014***
Biomechanical properties of the pediatric eye
Total Award:
Role: PI

Early Career Development Award ***PCMCF, 2012-2013***
Pediatric TBI from repetitive head rotation
Total Award:
Role: PI

University of Utah Research Foundation Seed Award ***URF, 2012-2013***
Quantification of collagen dissolution in the immature eye from plasmin protealase
Total Award:
Role: PI

National Center for Injury Prevention & Control (R01-CE001445) ***CDC, 2008-2012***
Development and validation of a diagnostic tool for infant head injuries from falls
Role: Multiple PI

University of Pennsylvania

National Center for Injury Prevention & Control (R01-CE001445) ***CDC, 2008-2012***
Development and validation of a diagnostic tool for infant head injuries from falls
Role: Multiple PI

National Institute of Neurological Disorders (Kirschstein-NRSA Trainee) ***NIH, 2008-2010***
Development of a novel model for repeated cyclic rotational brain injuries.

National Center for Injury Prevention & Control (Research Dissertation Grant) ***CDC, 2004-2006***
Mechanics of head impact in infants

Total Award:
Role: PI

SELECTED HONORS/DISTINCTIONS

2015	Mortar Board Honors Society Professor Award
2014	University of Utah nominee for the Blavatnik Young Scientist Award
2013	Teacher of the Year Award, Mechanical Engineering Department
2012	University of Utah nominee for the Packard Fellowship in Science and Engineering
2008	Recipient of David and Lindsay Morgenthaler Endowed Fellowship - Ophthalmology
2008	University Nominee for the Burroughs Wellcome Career Award at the Scientific Interface
2007	Solomon R Pollack Award for Excellence in Graduate Bioengineering Research
2001-2003	Stephenson Fellowship Award
2000-2004	Graduate Association of Bioengineers, President (2001-2003)
1999-2000	Clyde Christianson Scholarship
1999	Phi Beta Kappa, National Honor Society
1996	Tau Beta Pi, National Engineering Honor Society, Secretary (1997-1998)
1995-1999	Honors Scholarship

TEACHING EXPERIENCE

Tenure-Track Faculty ***University of Utah, 2010-Present***
Department of Mechanical Engineering Salt Lake City, UT
ME EN 1300: Statics and Introduction to Strength of Materials (Spring 2011, 2012, 2013, 2014)
ME EN 5510/6510: Introduction to Finite Element Modeling (Fall 2011, 2012, 2014, 2015)
ME EN 3300: Strength of Materials (Spring 2015)
ME EN 5540/6540: Biomechanics II (Spring 2016)
ME EN 5300/6300: Advanced Mechanics of Materials (Fall 2016)

Lecturer ***University of Pennsylvania, 2006-2008***
Department of Bioengineering Philadelphia, PA
BE 100: Introduction to Bioengineering (Fall 2006, 2007)
BE 200: Biomechanics and Biomaterials (Fall 2006, 2007)
BE 210: Sophomore Undergraduate Bioengineering Lab (Spring 2007, 2008)
BE 310: Junior Undergraduate Bioengineering Lab (Spring 2007, 2008)

Graduate Instructor of Record ***University of Pennsylvania, 2005-2006***
Department of Bioengineering Philadelphia, PA
BE 100: Introduction to Bioengineering (Fall 2005)
BE 210: Sophomore Undergraduate Bioengineering Lab (Spring 2006)
BE 310: Junior Undergraduate Bioengineering Lab (Spring 2006)

Teaching Assistant ***University of Pennsylvania, 2001-2004***
Department of Bioengineering Philadelphia, PA
BE 100: Introduction to Bioengineering (Fall 2004)
BE 200: Biomechanics and Biomaterials (Fall 2003)
BE 350: Transport Processes in Living Systems (Spring 2003)
BE 510: Biomechanics and Biotransport (Spring 2001)
BE 567: Modeling Biological Systems (Fall 2002)

SERVICE

University of Utah
Neuroscience Initiative Acquired Neural Injuries Steering Committee (2015-2016)

College of Engineering
Tau Beta Pi
- Advisor (2014-Present)

FE Exam Student Review
- Statics reviewer (Spring 2013)

Department of Mechanical Engineering
Department Executive Committee
- Member (2014-2016)

Solid Mechanics Group
- Chair (2014-2016)
- Member (2010-2014)

Distinguished Seminar Committee
- Chair (2012-2016)
- Member (2010-2012)

Strategic Planning Committee
- Member (2014-2016)

Graduate Committee
- Member (2014-2016)

Faculty Search Committees
- Chair (Computational Mechanics, 2013)
- Member (Computational Mechanics, 2011, 2012)
- Member (Experimental Mechanics, 2014)
- Member (Solid Mechanics, 2015)

Undergraduate Curriculum Committee
- Member (Spring 2014, Spring 2013)

Department of Pediatrics
Fellows Program
- Fellowship Advisor (2013-Present)

PROFESSIONAL SOCIETY MEMBERSHIPS

•American Society for Engineering Education (ASEE)
•Association for Research in Vision and Ophthalmology (ARVO)
•Association of Women in Science (AWIS)
•American Society of Mechanical Engineers (ASME)
•Biomedical Engineering Society (BMES)
•National Neurotrauma Society (NNS)
•Tau Beta Pi (TBP)

AD HOC REVIEWER

•Annals of Biomedical Engineering
•Archives of Pediatric and Adolescent Medicine

•ASME Journal of Biomechanical Engineering
•Biomechanics and Modeling in Mechanobiology
•Computer Methods and Programs in Biomedicine
•Experimental Eye Research
•International Journal of Numerical Methods
•Investigative Ophthalmology & Visual Science
•JAAPOS
•Journal of Biomechanics
•Journal of Clinical Anatomy
•Journal of Mechanical Behavior and Biomedical Materials
•Journal of Neurosurgery
•Medical and Biological Engineering and Computing
•Neurophotonics
•Pediatrics
•Traffic Injury Prevention

GRANT REVIEWER

Centers for Disease Control
National Center for Injury Prevention and Control (NCIPC)
Special Emphasis Panel (Fall 2009)

National Science Foundation
Nano and Biomechanics Program (Spring 2011)
Biomechanics and Mechanobiology (2012, 2015)

South Plains Foundation
Lubbock, TX (Summer 2011)

PUBLICATIONS

Book Chapters

1. Margulies SS and **Coats B**. Biomechanics of pediatric TBI. In V. Anderson & K. Yeates (Eds), *Pediatric Traumatic Brain Injury: New Frontiers in Clinical and Translational Research.* New York: Cambridge University Press. (2010)

2. Margulies SS and **Coats B.** Biomechanics of head trauma in infants and young children. In C. Jenny (Ed) *Child Abuse and Neglect: Diagnosis, Treatment and Evidence*. Philadelphia: Saunders/Elsevier. (2010)

3. Coats B and Margulies SS. Experimental injury biomechanics of the pediatric head. In J. Crandall, D. Meaney, B. Myers, and S. Zellers Schmidtke (Eds), *Pediatric Injury Biomechanics: Archive and Textbook*. New York: Springer. (2013)

4. Kleinman PK, **Coats B**, Silvera VM. Scalp, Subscalp and Cranium. In P. Kleinman (Ed.) *Diagnostic Imaging of Child Abuse.* 3rd Edition. Elsevier Science Health, St. Louis Missouri (2015)

5. Frasier L and **Coats B**. Clinical, Biomechanical, and Imaging Considerations. In P. Kleinman (Ed.) *Diagnostic Imaging of Child Abuse.* 3rd Edition. Elsevier Science Health, St. Louis Missouri (2015)

6. **Coats B** and Shedd DF. Biomechanics of eye injury in military scenarios. In. A. Gefen (Ed.) *Mechanobiology and Mechanophysiology of Military-Related Injuries.* New York: Spring. (2016)

7. Coats B and Margulies SS. Criminal Forensic Issues in Pediatrics. In B. Muller and S. Wolf (Eds.) *Handbook of Human Motion.* Springer International. Switzerland. *In preparation*

Peer-Reviewed Journal Publications
*Students of Coats B, *Mentorees* of Coats B

1. Prange MT, **Coats B**, Duhaime AC, and Margulies SS. Anthropomorphic simulations of falls shakes, and inflicted impacts for infants. J Neurosurg 2003, 99: 143-150

2. **Coats B** and Margulies SS. Characterization of pediatric porcine skull properties during impact, Proce Int Conf of Biomech of Impacts. 2003: pp. 57-66.

3. **Coats B** and Margulies SS**.** Material properties of porcine parietal cortex. J Biomech. 2006, 39(13):2521-5.

4. **Coats B** and Margulies SS. Material properties of human infant skull and suture at high rates. J. Neurotrauma. 2006 Aug, 23(8):1449-59.

5. **Coats B, Ji S** and Margulies SS. Parametric study of head impact in the infant. Stapp Car Crash Journal. 2007 Oct, 51:1-15.

6. **Coats B** and Margulies SS. Potential for head injury in infants from low height falls. J. Neurosurgery: Pediatrics. 2008 Nov, 2(11):321-30. **(Selected for journal cover and editorial feature)**

7. Margulies SS, **Coats B**, Christian C, Forbes B, Duhaime AC. What can we learn from computational model studies of the eye? JAAPOS. 2009 Aug; 13(4):332.

8. Ibrahim NG, Natesh R, Szczesny SE, Ryall K, Eucker SA, **Coats B**, Margulies SS. *In Situ* deformations in the immature brain during rapid rotations. J Biomechanical Eng. 2010 Apr; 132(4)

9. **Coats B**, Binenbaum G, Peiffer RL, Forbes BJ, Margulies SS. Ocular hemorrhages in neonatal porcine eyes from single, rapid rotational events. Investigative Ophthalmology & Visual Science (IOVS). 2010 Sept; 51(9)

10. **Coats B**, Eucker S, Sullivan S, Margulies SS. Finite element model predictions of intracranial hemorrhage from non-impact rapid head rotations in the piglet. International Journal of Developmental Neuroscience. Special Issue. 2012. Jan. 5

11. Moran P and **Coats B**. Biological sample preparation for SEM imaging of immature porcine retina. Microscopy Today. Jan 2012; 10-12.

12. *Tsai A*, **Coats B**, Kleinman PK. Stress profile of infant rib in the setting of child abuse: a finite element parametric study. Journal of Biomechanics. 2012 Jul; 45(11):1861-8.

13. Brooks BD, Sinclair KD, Davidoff SN, Lawson S, Williams AG, **Coats B**, Grainger DW, and Brooks AE. Molded polymer-coated composite bone void filler improves tobramycin controlled release kinetics. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2014 Jul; 102(5):1074-83.

14. Colter J, Williams AG, Moran P and **Coats B**. Age-related changes in dynamic moduli of ovine vitreous. Journal of Mechanical Behavior of Biomedical Materials. 2015 Jan; 41:315-324.

15. Payne A, de Bever J, *Farrer A*, **Coats B**, Parker DL, Christensen DA. A simulation technique for three-dimensional MR-guided acoustic radiation force imaging. Medical Physics. 2015 Feb; 42(2):674-784
16. Sullivan S, **Coats B**, and Margulies SS. Biofidelic neck influences head kinematics of parietal and occipital impacts following short falls in infants. Accident Analysis and Preventions. 2015 Jun; 82:143-153.
17. Scott GG and **Coats B**. Microstructural characterization of the pia-arachnoid complex using optical coherence tomography. IEEE Transactions on Medical Imaging. 2015 Jul; 34(7):1452-1459
18. Sullivan S, Eucker SA, Gabrieli D, Bradfield C, **Coats B**, Maltese MR, Lee J, Smith C, Margulies SS. White matter tract oriented deformation predicts traumatic axonal brain injury and reveals rotational direction-specific vulnerabilities. Biomechanics and Modeling in Mechanobiology. 2015 Aug; 14(4):877-96
19. *Li H*, Gehrke C, Sant H, **Coats B**, Agarwal J, Gale BK. A new vascular coupler design for end-to-end anastomosis: fabrication and proof-of-concept evaluation. ASME Journal of Medical Devices. 2015 Sept; 9(3):031002-6.
20. Farrer AI, Odeen H, de Bever J, **Coats B**, Parker DL, Payne A, Christensen DA. Characterization and evaluation of tissue-mimicking gelatin phantoms for use with MRgFus. Journal of Therapeutic Ultrasound. 2015 Jun; 3:9
21. *Li H*, Agarwal J, **Coats B**, Gale BK. Optimization and evaluation of a vascular coupling device for end-to-end anastomosis: a finite element analysis. Journal of Medical Devices. 2016 March; 10:011003-1-7.
22. Scott GG, Margulies SS and **Coats B**. Utilizing multiple scale models to improve predictions of extra-axial hemorrhage in the immature piglet. Biomechanics and Modeling in Mechanobiology. *Accepted October 2015. DOI: 10.1007/s10237-015-0747-0*
23. **Coats B**, Smith C, Binenbaum G, Peiffer RL, Christian CW, Duhaime AC, Margulies SS. Repeated episodes of cyclic head rotations produce modest brain injury in infant piglets. J Neurotrauma. *Accepted March 2016. DOI: 10.1089/neu.2015.4352*

Peer-Reviewed Journal Publications in Preparation

1. Colter J, Wirostko B, and **Coats B**. Finite-element design optimization of a hyaluronic acid-based hydrogel drug delivery device for use in the inferior fornix.
2. Shedd D, Jones J, Benko N and **Coats B**. Long-term changes in structure and function of the rat visual system after blast exposure.
3. Drysdale M, Kyoung H, Wirostko B, **Coats B**. Material property testing of a carboxymethylated hyaluronic acid hydrogel polymer for drug delivery to the eye.
4. Jones J, Shedd D, and **Coats B**. Proteomic changes in the vitreous following blast exposure.
5. Benko N, Shedd D, and **Coats B**. Intraocular pressure changes following blast exposure in rats.
6. Evans SM, Smith C and **Coats B**. The physiological effect of crying on repetitive head trauma. Journal of Pediatrics
7. Saffioti JM and **Coats B**. Age, region and strain dependent material properties of the ovine sclera. Journal of Mechanical Behavior of Biomedical Materials.

8. Saffioti JM and **Coats B**. Characterizing the effect of post-mortem time and storage condition on the mechanical properties of immature and adult ovine sclera. J. Biomechanics
9. Binenbaum G, Evans SM, and **Coats B**. Rapid decompression of the eye as a mechanism for retinal hemorrhage. Journal of Ophthalmology
10. Binenbaum G, Evans SM, McMillan KR, and **Coats B**. Retinal vein occlusion as a mechanism for retinal hemorrhage. Experimental Eye

Conference Podium Presentations – invited presentations excluded

1. **Coats B** and Margulies SS. Characterization of pediatric porcine skull properties during impact, Proce Int Conf of Biomech of Impacts. 2003: pp. 57-66.
2. **Coats B,** Ji S and Margulies SS. Parametric study of head impact in the infant. Stapp Car Crash Journal. 2007 Oct, 51:1-15.
3. **Coats B**, Ji S, Margulies SS. Using computational models to predict skull fracture in the infant. Pediatric Abusive Head Trauma, Hershey, PA, July 2007
4. **Coats B**, Binenbaum G, Pieffer RL, Forbes BJ, Margulies SS. Ocular hemorrhages from rapid, rotational accelerations. Association for Research in Vision and Ophthalmology, Ft. Lauderdale, FL, May 2009
5. **Coats B**, Binenbaum G, Pieffer RL, Forbes BJ, Margulies SS. Ocular hemorrhage in single, non-impact head rotations: a porcine model. Pediatric Abusive Head Trauma Conference, Jackson Hole, WY, June 2009
6. Herman BE, **Coats B**, Corwin DL. Detailed confessions and reenactments utilizing biomechanical dolls: What do they tell us? Pediatric Abusive Head Trauma Conference, San Francisco, CA. July 2011.
7. **Coats B**, Corwin D, Herman B. Video recorded reenactment of inflicted head and other injuries to a three-week old infant: biomechanical, clinical and psychosocial observations and perspectives. International Conference on Child & Family Maltreatment, San Diego, CA. January 2012
8. **Coats B** and Binenbaum G. Progress toward understanding mechanisms of retinal hemorrhage. 12th International Conference on SBS/AHT. Boston, MA September 2012.
9. Scott GG and **Coats B**. Microscale finite element model of the pia arachnoid complex in shear: a parametric evaluation. Proceedings of the 11th International Symposium of Computer Methods in Biomechanics and Biomedical Engineering. Salt Lake City, UT. April 2014.
10. Evans SM, Smith C and **Coats B**. The effect of physiological changes due to crying on repeated pediatric head trauma. 17th U.S. National Congress on Theoretical and Applied Mechanics. East Lansing, MI. June 2014
11. Scott GG and **Coats B**. Microscale finite element modeling and optical coherence tomography imaging of the pia arachnoid complex. 17th U.S. National Congress on Theoretical and Applied Mechanics. East Lansing, MI. June 2014.
12. Shedd DF and **Coats B**. Visual dysfunction following low level blast exposure in rats. ARVO. Denver, CO May 2015
13. *Gomez AD*, Scott GG, Terry BC, **Coats B**. Image-based dynamic analysis of brain deformation model. Summer Biomechanics, Bioengineering and Biotransport Conference. Snowbird, UT June 2015.

14. Shedd DF, Jones JA, *Zaugg B*, **Coats B**. Cornea damage progression following blast exposure. Summer Biomechanics, Bioengineering and Biotransport Conference. Snowbird, UT June 2015. **PhD Student Paper Finalist**

15. McMillan KR, Evans SM, Binenbaum G, **Coats B**. Measuring tortuosity changes due to central retinal vein occlusion. Abusive Head Trauma Conference. Park City, UT July 2015

16. Terry BC, Scott GG, Abdullah O, **Coats B**. DTI voxel-wise analysis of porcine mild TBI. Abusive Head Trauma Conference. Park City, UT July 2015.

17. *Tsai T*, **Coats B**, Kleinman P. Finite element analysis of the classic metaphyseal lesion of infant abuse: a preliminary experience. International Pediatric Radiology Conjoint Meeting & Exhibition. Chicago, IL. May 2016

Conference Poster Presentations

1. Prange MT, **Coats B**, Raghupathi R, Duhaime AC, Margulies SS. Rotational Loads During Inflicted and Accidental Infant Head Injury, National Neurotrauma Symposium, San Diego CA. November 2001.

2. **Coats B** and Margulies SS**.** Material properties of porcine parietal cortex. World Cong. on Biomech. Aug. 2002.

3. **Coats B** and Margulies SS. Mechanical properties of human pediatric skull at high impact rates. ASME Summer Bioeng Conference, Vail, CO, June 2005. **Student Paper Competition Finalist**

4. **Coats B** and Margulies SS. High rate material properties of infant cranial bone and suture. National Neurotrauma Symposium, Washington DC, November 2005.

5. Ibrahim NG, **Coats B**, and Margulies SS. Response of the toddler and infant head during shaking. National Neurotrauma Symposium, Washington DC, November 2005.

6. **Coats B**, Ji S, Margulies SS. Using computational models to predict skull fracture in the infant. Pediatric Abusive Head Trauma, Hershey, PA, July 2007

7. Ibrahim NG, **Coats B**, and Margulies SS. Kinematics of the infant and toddler head during low height falls. National Neurotrauma Symposium, Florida, July 2008

8. **Coats B**, Binenbaum G, Pieffer RL, Forbes BJ, Margulies SS. Ocular hemorrhages from rapid, rotational accelerations. Association for Research in Vision and Ophthalmology, Ft. Lauderdale, FL, May 2009

9. **Coats B**, Kras J, Eucker, S, Margulies SS. Severity of extra-axial hemorrhage from non-impact inertial head rotation in the immature pig varies with direction. Neurotrauma, Santa Barbara, CA, September 2009

10. **Coats B**, Sullivan S, and Margulies SS. Development of a finite element model for predicting subdural hemorrhage from rapid non-impact head rotations. Neurotrauma, Las Vegas, NV, June 2010.

11. **Coats B**, Binenbaum G, Peiffer RL, Sullivan S, Ralston J, Smith C, Duhaime AC, Margulies SS. Ocular and Neuropathology from repetitive, low-velocity head rotations in immature pigs. Neurotrauma, Fort Lauderdale, FL, July 2011

12. Moran P. and **Coats B**. Development of rheometry tools for preventing wall slip in immature porcine vitreous. 7th Annual Utah Biomedical Engineering Conference. Salt Lake City, UT, September 2011.

13. Williams A, Nelson N, Abbott J and **Coats B**. Finite element analysis of a magnetically driven screw through soft tissue. 7th Annual Utah Biomedical Engineering Conference. Salt Lake City, UT, September 2011

14. Hashmi SK, Sullivan S, Eucker SA, **Coats B**, Lee J, Margulies SS. FEM-predicted regional tissue strains aligned with the white matter tracts predict axonal injury. Northeast 38th Annual Bioengineering Conference (NEBEC). Philadelphia, PA. March 2012

15. Moran P. and **Coats B**. A rheological technique for extracting dynamic moduli from creep testing of pediatric porcine vitreous. Proceedings of the ASME 2012 Summer Bioengineering Conference Fajardo, Puerto Rico, June 2012. **MS Student Paper Finalist**

16. Saffiotti JM and **Coats B**. Development of a finite element model for intraocular pressure predictions in children. Proceedings of the 11th International Symposium of Computer Methods in Biomechanics and Biomedical Engineering. Salt Lake City, UT. April 2013.

17. Shedd DF and **Coats B**. Verification of a shock tube finite element model for blast ocular injury simulations. Proceedings of the 11th International Symposium of Computer Methods in Biomechanics and Biomedical Engineering. Salt Lake City, UT. April 2013.

18. Saffioti JM and **Coats B**. Age dependent and anisotropic material propertis of immature porcine sclera. Proceedings of the ASME 2013 Summer Bioengineering Conference. Sun River, Oregon. June 2013.

19. Farrer AI, de Bever J, **Coats B**, Christensen DA, Payne A. Fabrication and evaluation of tissue-mimicking gelatin phantoms for use with MR-ARFI and MRgFUS. 14th International Symposium on Therapeutic Ultrasound. Las Vegas, NV. April 2014.

20. Shedd DF and **Coats B**. Temporary visual dysfunction following low-level blast exposure. 7th World Congress of Bomechanics. Boston, MA. July 2014.

22. Saffioti JM and **Coats B**. The effect of post mortem time on the material properties of immature and adult ovine sclera. 7th World Congress of Biomechanics. Boston, MA July 2014.

23. Colter JC, Williams A, Moran P, **Coats B**. Dynamic moduli of immature ovine vitreous using advanced rheology techniques. 7th World Congress of Biomechanics. Boston, MA July 2014. **BS Student Finalist**

23. Evans SM, Smith C and **Coats B**. The effect of physiological changes due to crying on repeated pediatric head trauma. 7th World Congress of Biomechanics. Boston, MA July 2014.

24. *Farrer A*, Odeen H, de Bever J ,**Coats B**, Christensen D, Payne A. Development and characterization of tissue-mimicking gelatin phantoms for use with MRgFUS. Biomedical Engineering Society. San Antonio, TX Oct 2014

25. Binenbaum G, Evans S, Lee V, **Coats B**. Evaluation of optic nerve compression as a potential cause of retinal hemorrhage in infants. AAPOS 41st Annual Meeting. New Orleans, LA March 2015

26. **Coats B**, Drysdale M, Lee HK, Wirostko BM. Mechanical properties of four carboxymethylated hyaluronic acid hydrogel polymer formulations. ARVO. Denver, CO May 2015

27. Lee HK, Onorato M, Erickson I, Rafii MJ, **Coats B**, Zarembinski T, Wirostko BM. Novel carboxymethylated hyaluronic acid drug delivery polymer for repair of ocular injuries. ARVO. Denver, CO May 2015.

28. Drysdale M, Lee HK, Wirostko B, **Coats B**. Material property testing of carboxymethylated hyaluronic acid hydrogel polymer. Summer Biomechanics, Bioengineering and Biotransport Conference. Snowbird, UT June 2015. **2nd Place in BS Student Paper Competition**

29. Jones JA, Shedd DF, **Coats B**. The temporal change in protein biomarkers in the vitreous humor following blast trauma. Summer Biomechanics, Bioengineering and Biotransport Conference. Snowbird, UT June 2015. **3rd Place in BS Student Paper Competition**

30. McMillan KR, Evans S, Binenbaum G, **Coats B**. Measuring tortuosity changes due to central retinal vein occlusion. Summer Biomechanics, Bioengineering and Biotransport Conference. Snowbird, UT June 2015.

31. Colter J, Cady N, Lee HK, Mann B, Wirostko B, **Coats B**. Design optimization to improve retention of a carboxymethylated hyaluronic acid (CMHA-S) drug delivery device. ARVO. Seattle, WA. May 2016

32. Jones J, Shedd D, **Coats B**. Temporal changes in vitreous inflammatory cytokines and neurofilament heavy chain following ocular trauma. ARVO. Seattle, WA. May 2016

33. Creveling CJ and **Coats B**. An innovative method for measuring adhesion at the vitreoretinal interface. Summer Biomechanics, Bioengineering and Biotransport Conference. National Harbor, MD. June 2016

34. Terry BC, Scott GG, Abdulla O, **Coats B**. DTI voxel-wise analysis of mild TBI in neonatal pigs following non-impact head rotation. Summer Biomechanics, Bioengineering and Biotransport Conference. National Harbor, MD. June 2016

INVITED TALKS

*expenses paid by host

Basics: Biomechanics of Abusive Head Trauma. 15th International Conference on SBS/AHT. Montreal, CA. September 2016*

Using Finite Element Analysis to Improve Deformation Calculation in MRI Imaging. Computer Methods in Biomechanics and Biomedical Engineering. Tel Aviv, Israel. September 2016.

Temporal progression of ocular pathology following primary blast exposure. Ophthalmology Grand Rounds. Moran Eye Center. University of Utah. April 2016.

Predicting traumatic brain injury. Department of Materials Science. University of Utah. Salt Lake City, UT March 23.

The importance of microstructure in predictions of brain injury. Neurotrauma Masters Conference. Salt Lake City, UT. March 2016

Predictions of intracranial hemorrhage using a subject-specific multiscale model of the pia-arachnoid complex. Computer Methods in Biomechanics and Biomedical Engineering. Montreal, Canada. September 2015

Biomechanics of retinal injuries. Abusive Head Trauma Conference. Park City, UT. July 2015*

The effect of the volume fraction and damage of arachnoid trabeculae on traumatic brain injury. Neuroscience Grand Rounds. Department of Neuroscience. University of Manitoba, Winnipeg. Canada. October 2014*

The mechanics of arachnoid trabeculae and their influence on traumatic brain injury: a multiscale investigation. Department of Biomedical Engineering. University of Manitoba, Winnipeg. Canada. October 2014*

Developing a better method of understanding SBS through biomechanical and pathological research. 14th International Conference on SBS/AHT. Denver, CO. September 2014*

Understanding mechanisms of retinal hemorrhage through experimentation, mechanical testing, and imaging. 14th International Conference on SBS/AHT. Denver, CO. September 2014*

Microscale finite element modeling and optical coherence tomography imaging of the pia arachnoid complex. 7th World Congress of Biomechanics. Boston, MA. June 2014.

Modern research on retinal hemorrhages in abusive head trauma. Abusive Head Trauma Conference. Burlington, VT. June 2013* (declined due to scheduling conflict)

Finite element modeling in research and education. ANSYS Medical Device Forum. Salt Lake City, UT. October 2012*

Progress toward understanding mechanisms of retinal hemorrhage. 12th International Conference on SBS/AHT. Boston, MA. September 2012*

Predicting injury in pediatric head and eye trauma: Combining simulations and databases. Department of Biomedical Informatics, University of Utah. Salt Lake City, UT. September 2012

Abusive head trauma: a case, a confession, and the biomechanics involved. Trauma Research and Education Meeting. Trauma Department at Primary Children's Medical Center. Salt Lake City, UT. December 2010

Using biomechanics to understand SBS/AHT. 11th International Conference on SBS/AHT. Atlanta, GA. September 2010.*

What biomechanics has taught us about pediatric TBI. Ground Rounds Seminar. Department of Pediatrics at Primary Children's Hospital. Salt Lake City, UT. February 2010.*

Using biomechanics to elucidate mechanisms of retinal hemorrhages in children. Ground Rounds Seminar. Department of Ophthalmology at Moran Eye Center. Salt Lake City, UT. October 2009.*

Biomechanics of pediatric head and eye injury in accidental and inflicted trauma. Department of Mechanical Engineering at University of Utah. Salt Lake City, UT. July 2009.

Ocular hemorrhages in single, non-impact had rotations: a porcine model. Penn State College of Medicine. Jackson Hole, WY. June 2009.

Biomechanics of brain, skull, and eye injury in abusive head trauma. Cole Eye Institute and Cleveland Clinic Lerner Research Institute. Cleveland, OH. December 2008.*

Mechanics of head impact in infants. CNS Seminar. Department of Neurosurgery at the Hospital of the University of Pennsylvania. Philadelphia, PA. September 2007.

Biomechanics in pediatric head injury. Summer Academy in Applied Science and Technology. Philadelphia, PA. August 2007

Using computational models to predict skull fracture in the infant. Penn State College of Medicine. Hershey, PA. July 2007.

Head injury biomechanics in infants. Department of Mechanical Engineering at Virginia Tech. Blacksburg, VA. March 2007.*

Predictions of skull fracture in infants. Center for Brain Injury Research. Philadelphia, PA. November 2006.*

Mechanics of head impact in infants. CDC New Investigator Workshop. Atlanta, GA. May 2005.*

Characterization of pediatric porcine skull properties during impact. International Research Council on the Biomechanics of Injury. Lisbon, Portugal. September 2003.

Temporal Progression of Visual Injury from Blast Exposure

Proposal Number: 11257006
Award Number: W81XWH-12-1-0243

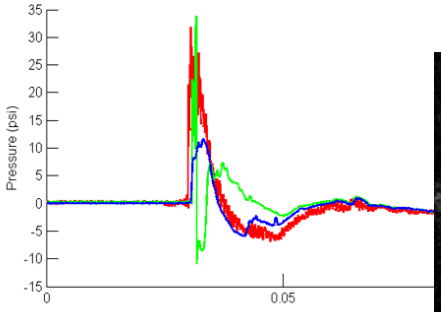
PI: Brittany Coats **Org:** University of Utah **Award Amount:** $997,528

Study/Product Aim(s)

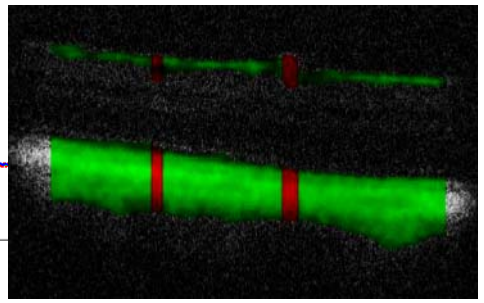
- Investigate progression of visual system injury in service members exposed to blast
- Investigate progression of visual system injury following blast exposure in an animal model and identify early indicators of visual dysfunction
- Identify changes in vitreous protein expression that correlate with visual system injury

Approach

1) Retrospective chart review of service members exposed to blast as well as prospective study.
2) Track visual acuity in rat model with optokinetic testing and OCT subsequent to blast injury simulated by shock tube.
3) Collect vitreous samples following animal studies; assay for NfH, VEGF, IL-10, MCP-1, MIP-3.



IOP Cables



GC/NFL Thickness

Accomplishments: Completed IOP studies, typical result shown above. Retina measurements and analysis completed.

Timeline and Cost

Activities FY	13	14	15	16
Investigate the progression of visual system injury in service members exposed to blast				
Investigate the progression of visual system injury following blast exposure in animal model				
Identify changes in vitreous protein expression that correlate with visual system injury				
Estimated Budget ($K)	301	239	266	191

Updated: September 30, 2016

Goals/Milestones

CY13 Goal – IRB/IACUC Approvals, system acquisition, initial testing
☑ Obtain equipment required for experimental setup
☑ Get IRB and IACUC approvals Identify service members exposed to blast between 2007-12
☑ Complete first set of 40 animal blast experiments
CY14 Goals – Animal testing, service member studies
☑ Complete data analysis from retrospective study
☐ Enrollment, interviews, and ocular examination of service members
☑ Complete animal blast experiments
☑ Complete first set of protein assays
CY15 Goal – Data analysis, prospective studies
☐ Complete enrollment of service members for prospective studies
☑ Complete data analysis for experimental studies, protein assays
CY16 Goal – Clinical ocular exams, data analysis
☐ Complete clinical exams for participants and data analysis
☑ Complete data analysis of protein assays

Comments/Challenges/Issues/Concerns: IOP sensors out for calibration and maintenance after potential damage from blast studies. IOP studies paused until they return in July.

Budget Expenditure to date
Projected Expenditure: 949,250 Actual Expenditure: 852,841